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**DISCOVERY AND DESCRIPTION OF THE HOSPICIDAL
FIRST INSTAR OF *EPEOLUS AMERICANUS* (CRESSON)
(HYMENOPTERA: APIDAE), A CLEPTOPARASITE
OF *COLLETES CONSORS MESOCOPUS* SWENK
(HYMENOPTERA: COLLETIDAE)**

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Abstract

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A host-cleptoparasite association is newly confirmed between the solitary bee *Colletes consors mesocopus* Swenk (Hymenoptera: Colletidae) and its cuckoo bee *Epeolus americanus* (Cresson) (Hymenoptera: Apidae). Females of *E. americanus* were observed entering the nests of *C. consors mesocopus* in June 2020 at a clay pit in Meadow Creek, British Columbia. Unearthed brood cells revealed four *Epeolus* first instars, one of which was DNA barcoded and thereby identified as *E. americanus*. The first instar of this species is for the first time described and imaged. Additionally, observations of nest-seeking behavior by *E. americanus*, a description of the host nest architecture, and brood cell contents are presented and discussed in the context of what is known from studies of other species of *Colletes* and *Epeolus*.

Introduction

The bee genus *Epeolus* Latreille (Hymenoptera: Apidae) consists exclusively of (insofar as is known) cleptoparasites of polyester (or cellophane) bees of the genus *Colletes* Latreille (Hymenoptera: Colletidae) (Onufenko *et al.* 2019). As in all members of the entirely cleptoparasitic subfamily Nomadinae *sensu* Michener (2007), the first instars of *Epeolus* are hospicidal and characterized by long sickle-shaped mandibles, long (presumably sensory) labral tubercles, a prognathous and elongate sclerotized head capsule, and a pygopod to facilitate mobility (Rozen 1989; Michener 2007). Together, these features enable the cleptoparasitic bee larva to seek out and kill the solitary host bee's egg or larva within the closed brood cell (as well as any eggs or larvae of conspecifics in the cell if it has been superparasitized), after which it proceeds to feed and develop on the pollen-nectar

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provisions of its host. These morphological adaptations are lost in subsequent stages of development, with Torchio and Burdick (1988) reporting for one species of *Epeolus* (*E. compactus* Cresson) the disappearance of the pygopod and lateral body tubercles as well as a reduction in the size of the mandibles, labral tubercles, and maxillary palpi by the second instar. Third and subsequent instars resemble those of non-parasitic bees and are characterized by a hypognathous rounded head capsule, and the mandibles, labral tubercles, and maxillary palpi are more strongly reduced in size (Torchio and Burdick 1988). *Epeolus* and other nomadines thus undergo hypermetamorphosis (Wheeler 1919).

Although *Epeolus* is currently represented by 43 valid species in North America north of Mexico (Onufko 2018), the immature stages of only three species (*E. compactus*, *E. ilicis* Mitchell, and *E. pusillus* Cresson) were known, described, and vouchered before the present study (Rozen 1989). The larvae of cleptoparasitic bees, and especially the homicidal first instars, are rarely collected compared with adults due to their confinement to the host brood cell, which precludes their collection by means of passive sampling, the difficulty of finding parasitized host nests, and their rapid development (first instars of *E. compactus* for example molt into second instars between one and two days after egg eclosion; Torchio and Burdick 1988). Therefore, studies of the immature stages of *Epeolus* are limited to a few species whose larvae were discovered opportunistically in parasitized host nests. As a result, most reported host-cleptoparasite associations between *Colletes* and *Epeolus* are based not on nest excavations but rather on observations of adults of a particular species of the latter collected at the same place and time as adults of a particular species of the former and the presumption that *Colletes* is the only genus that serves as a host for *Epeolus* (Onufko 2018; Onufko *et al.* 2019). The presumption is based on the morphology of the sixth sternum (S6) of the adult female of *Epeolus*. Uniquely among the eight genera comprising the tribe Epeolini, the S6 of the female of *Epeolus* is modified to cut or punch a hole through the polyester cell wall lining and/or cell cap of the *Colletes* host brood cells, through which *Epeolus* females lay and conceal their eggs (Rightmyer 2004). Nevertheless, associations based on observations of co-occurrence may be coincidental and to be confirmed require observations of *Epeolus* larvae or ovipositing *Epeolus* adult females within the host nest (Wolf and Ascher 2009; Onufko *et al.* 2019).

The purpose of the present study is threefold: 1) to provide definitive evidence of a host-cleptoparasite association between *Colletes consors mesocopus* Swenk and *Epeolus americanus* (Cresson), confirmed by the discovery of the larvae of the latter in closed, provisioned brood cells of the former; 2) to describe and image for the first time the first instar of *E. americanus*, a species that is widespread in North America; and 3) to present information about the nesting habits of both species in the context of what is known about the nesting habits of their congeners.

Materials and methods

In mid-June 2020, an aggregation of *Colletes* nests was discovered by the second author in the Kootenay Region of British Columbia, approximately two kilometers north of the town of Meadow Creek (50.253 °N; 116.984 °W). At the site, which is a clay pit 639 m.a.s.l., 46 *Colletes* brood cells were unearthed with a trowel on 27 June 2020, around when

nesting activities seemed to be at their peak. The brood cells, which were quite robust and remained intact upon excavation because of their cellophane-like lining, were placed in an open container and transported to the second author's residence, where they were carefully opened with a pair of fine forceps. All larvae, including four *Epeolus* first instars, were euthanized by immersion in 80% ethanol and stored in ethanol-filled glass vials in a freezer. A few cells contained unexpected lodgers—ants (in three cells) and a small spider (in a cell that contained a bee egg)—which were also preserved. Two other cells that contained only bee eggs were left alone to allow the larvae to hatch, but they failed to do so and thus were ultimately discarded.

The ethanol-preserved specimens and a subset of the opened brood cells, which were stored in the same vials as their contents, were sent to the first author for further study at the Canadian Museum of Nature (CMN) Natural Heritage Campus in Gatineau, Quebec. Additionally, five *Epeolus* and six *Colletes* adults collected by the second author at the nesting site between 16 June 2020 and 25 June 2020 were sent to the first author for identification (one in ethanol, the rest as pinned, dried specimens). An adult female and male of each species are deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes in Ottawa, Ontario (CNC). Depositories for non-barcoded *E. americanus* first instars include the American Museum of Natural History in New York, New York (AMNH), CNC, and Packer Collection at York University, Toronto, Ontario (PCYU).

Morphological studies

Adults were examined under a standard dissecting microscope and identified to species/subspecies following the taxon concepts (Meier 2017; Packer *et al.* 2018) of Stephen (1954) for *Colletes* and Onufeko (2018) for *Epeolus*. Identifications of adult male *Colletes* (three specimens) involved excision and examination of the (mostly if not entirely) hidden seventh sternum, the morphology of which is highly variable among species and thus usually diagnostic. To study the immature stages, specimens were transferred to a glass plate containing enough ethanol to completely cover them, and high-resolution color images were taken using the Leica Z16 APO A imaging system (Leica Microsystems, Wetzlar, Germany) and focus-stacked using the accompanying LAS software. Images were minimally edited in PaintShop Pro (Jasc Software, Inc.) and assembled into figure plates and labelled in Adobe Photoshop 2020 (Adobe Inc.).

The description of the *E. americanus* first instar generally follows the format of Rozen (1989) and only includes features that are variable among the species of Epeolini for which first instars are known. Specimens were described from detailed images except measurements of anatomical features were taken using a standard dissecting microscope with an eyepiece graticule and calibrated with a stage micrometer.

DNA barcoding

To verify the identities of the sampled *Colletes* and *Epeolus* larvae, DNA was extracted from a subset of specimens at the CMN Laboratory of Molecular Biodiversity. A protocol for animal DNA extraction adapted from Ivanova *et al.* (2006) was followed. Due to their small size, entire larvae were immersed in lysis buffer. Apart from the only barcoded *Epeolus* first instar, which was preserved as its sclerotized head capsule remained intact following DNA extraction, barcoded larvae were largely disintegrated by lysis and thus

discarded, so images thereof were taken before extraction to serve as vouchers. A single *Colletes* adult female selected for barcoding was soaked in 80% ethanol for several hours, after which the head, prosternum, propleura, and fore legs were detached, and softened muscle tissues (the target source for genetic material) were removed from the mesosoma with a pair of fine forceps. The specimen was subsequently repinned, and the head and detached thoracic structures were reattached to the rest of the body using Elmer's Washable Clear School Glue.

The universal primers LepF1 / LepR1 (Hebert *et al.* 2004) were used to amplify a 658 bp segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, dubbed the DNA barcode (Hebert *et al.* 2003a, b). The 15 µL PCR reaction mixture consisted of 7.05–9.05 µL of DNA-grade water, 3 µL of 5 × Q5® Reaction Buffer (New England Biolabs (NEB), Ipswich, Massachusetts, USA), 0.3 µL of dNTPs (10 mM), 0.75 µL of forward primer (10 µM), 0.75 µL of reverse primer (10 µM), 0.15 µL of Q5® High-Fidelity DNA polymerase (0.3 U/rxn) (NEB), and 1–3 µL of DNA template. The following PCR conditions, which were programmed into an Eppendorf® Mastercycler® Pro Thermal Cycler, were used: 1 minute at 94 °C (initial denaturation); five cycles of 40 s at 94 °C (denaturation), 40 s at 45 °C (annealing), and 1 min at 72 °C (extension); 35 cycles of 40 s at 94 °C (denaturation), 40 s at 51 °C (annealing), and 1 min at 72 °C (extension); 5 min at 72 °C (final extension).

Sanger sequencing in both directions was carried out using the same primers used in PCR. The protocol used in the present study for the sequencing reaction, purification of PCR products, and automated ABI sequencer runs is detailed in Onufko *et al.* (2019).

Trace files were imported into Geneious 11.1.5 (Kearse *et al.* 2012) for assembly into consensus sequences, for quality checks, and to ensure that no stop codons were present in the correct reading frame. To validate our identifications, FASTA-formatted sequences were individually entered into the Barcode of Life Data System (BOLD) Identification Engine (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) for comparison to sequences in the BOLD database. This enabled us to identify the molecular operational taxonomic units to which the new sequences belong, denoted by separate barcode index numbers (BINs), which usually correspond to real species (Ratnasingham and Hebert 2007, 2013). Using the Geneious (multiple) Alignment algorithm, sequences were aligned into a matrix that included previously published and publicly available sequences of *Epeolus* (sample IDs: CCDB - 01565 G5, CCDB-24580 B09; Onufko 2018) and *Colletes* (sample IDs: LRBBC2713, KJH.86) from BOLD as well as all new sequences. A neighbor-joining tree based on the Tamura-Nei genetic distance model (Tamura and Nei 1993) was generated to visualize genetic distances among larvae and associated adults and determine whether they belong to the same species. All newly generated sequences are deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) under the accession numbers MW174769–MW174775.

Results

Nest site observations

Throughout the second half of June 2020, large numbers of *Colletes* adults were observed at the Meadow Creek nest site. There was so much activity that the sound made

by the rapid wingbeats of bees could be heard throughout the nesting area. Those caught in an insect net at the site and pinned (six specimens) were all identified as *Colletes cossors mesocopus*. The species appeared to be foraging mainly on introduced yellow hawkweed, *Pilosella caespitosa* (Dumort.) P.D.Sell & C.West (Asteraceae) (Fig. 1A, B). Many males of *Epeolus americanus* were present at the same site throughout this period, seen visiting mainly flowers of introduced oxeye daisy, *Leucanthemum vulgare* (Vaill.) Lam. (Asteraceae). Several *E. americanus* males were captured by crab spiders (Araneae: Thomisidae) on the flowerheads of *L. vulgare*. Females of *E. americanus* were also observed, some crawling on the ground and investigating *Colletes* nest entrances. Observations of nesting activity were made on cloudless or mainly sunny days, with the ambient temperature ranging from 24 °C to 28 °C.

On 22 June 2020 between 17:30 and 18:00, three visits by *E. americanus* females, each to a separate nest, were observed, lasting 13 s, 15 s, and 18 s, respectively. A few *E. americanus* females were seen being repelled from nest entrances by guarding *Colletes* females. The entire aggregation of *Colletes* nests covered an area of about 7.5 m × 3–4 m.

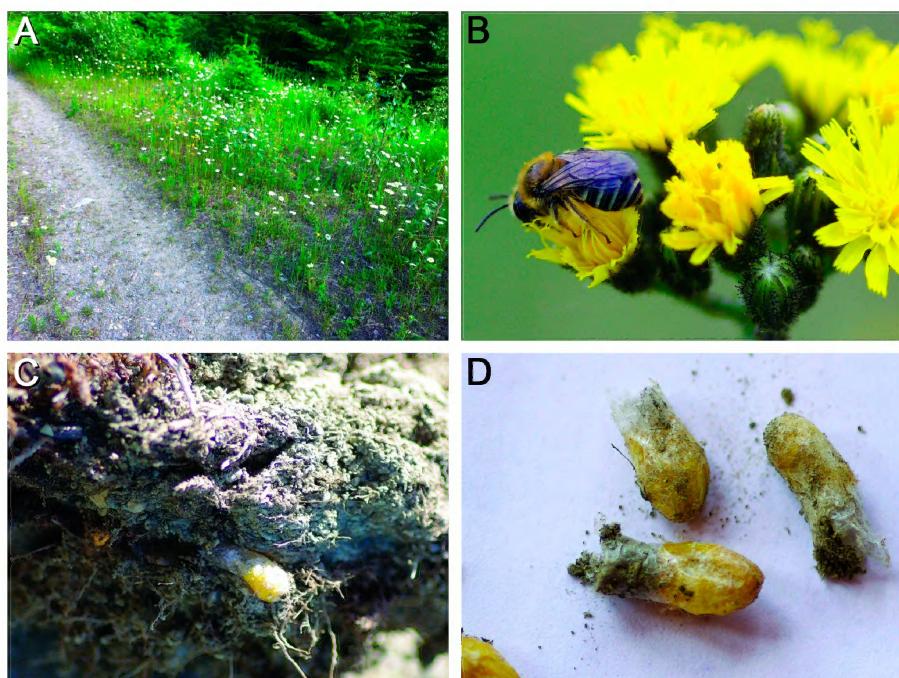


FIGURE 1. Nest site observations: (A) habitat, wherein the dominant flowers were *Leucanthemum vulgare* and *Pilosella caespitosa*; (B) female *Colletes* at flower head of *P. caespitosa*; (C) exposed brood cell and tunnel of *C. cossors mesocopus* nest; (D) unearthed soil-plugged brood cells of *C. cossors mesocopus* containing large stores of yellow semiliquid pollen-nectar provisions.

Many *Colletes* females were observed entering and exiting nests whereas males were flying low over the entire area. There were generally between eight and twelve nest entrances in a 50 cm × 50 cm area, and most nests were on level ground.

On 23 June 2020 between 11:40 and 12:20, one visit by an *E. americanus* female to a nest was observed that lasted 58 s. A female was also observed entering eight separate nest entrances, with visits lasting between 2 s and 13 s, before departing to feed on the flowers of a *Medicago falcata* L. (Fabaceae) plant nearby. Another female was followed and observed inspecting the entrances to nine nests in what appeared to be a random pattern, with visits lasting from 1 s to 4 s, and followed by a brief pause before resuming the process of investigating nests of potential hosts. At one point, there appeared to be a tussle among three females of *E. americanus* that came into close proximity with one another while searching for *Colletes* nests. Other bees that were present in the area at this time included members of the genera *Andrena* Fabricius (Hymenoptera: Andrenidae), *Lasioglossum* Curtis (Hymenoptera: Halictidae), and *Sphecodes* Latreille (Hymenoptera: Halictidae).

On 1 July 2020, no *E. americanus* adults were found at the nesting site, and substantially fewer *Colletes* adults were observed in the area.

Host nest architecture

Brood cells were located at depths ranging from 4 to 7 cm below the surface and linked to the nest entrances by unlined tunnels (Fig. 1C). Due to the very high concentration of brood cells in the soil, it was unclear how many were linked to a single nest entrance. Nest entrances did not have tumuli. Digging through the gravelly/sandy soil also revealed older, disused brood cells among the sealed newly provisioned cells.

The capped brood cells were somewhat cylindrical in shape, with an approximate length of 16 mm (10–11 mm excluding the soil-filled cup forming the plug) and diameter of 6–7 mm, which was greatest in the distal region beyond the “neck” of the cell (Fig. 1D). This region was filled with semiliquid pollen-nectar provisions, leaving a small unprovisioned area near the cell cap. In two cells, an egg, presumably that of *C. cossors mesocopus*, was discovered in this unprovisioned region. The entire cell wall was comprised of a thick cellophane-like lining of two or perhaps more layers, of which the outermost layer came off in thin sheets when pulled away from the thicker inner lining by fine forceps.

Nest inhabitants

All collected closed, provisioned brood cells were opened to reveal their contents. Most contained single C-shaped larvae, identified as *C. cossors mesocopus* (in 33 brood cells) based on DNA barcodes obtained from a subset of preserved specimens (N = 3; sample IDs: CMNTO_039, CMNTO_043, CMNTO_046). Larval sequences clustered closely with the sequence of the newly barcoded adult female (sample ID: CMNTO_041; see Fig. 2) and all were matched with 100% similarity to a sequence assigned the BIN BOLD:ABY8451. Three cells each contained a single highly mobile *Epeorus* first instar, one of which was barcoded and its sequence matched with 98.6% similarity to a sequence of *E. americanus* on BOLD (BIN: BOLD:AAB9110). Two cells each contained two unhatched eggs: one within the brood cell and a smaller egg (approximately half the size of the larger one) apparently on the outside. Since these eggs failed to hatch, we were unable to determine the species to which they belong; however, based on their location within the brood cells, the larger

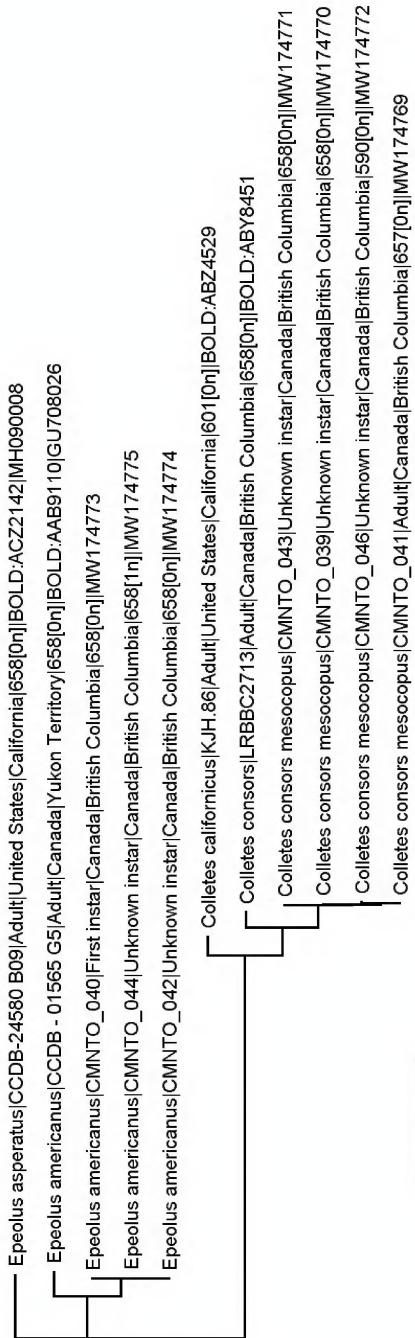


FIGURE 2. A neighbor-joining tree of *Epeorus* and *Colletes* COI sequences ≥ 590 bp in length, generated in Geneious and based on the Tamura-Nei genetic distance model. Included are previously published and publicly available reference sequences of *E. americanus* and *C. consors* from BOLD as well as sequences of members of the nearest BINs, BOLD:ACZ2142 and BOLD:ABZ4529 respectively. Sequence length is indicated for each sample, along with the number of ambiguous bases (in square brackets). Additionally, GenBank accession numbers (indicated by a two-letter alphabetical prefix followed by six digits) are given for sequences deposited in GenBank. Scale bar = 5% pairwise distance.

eggs were presumed to be those of *C. consors mesocopus*. Two other cells each contained two larvae. In one of the two cells, there was an *Epeorus* first instar and a larger C-shaped *Colletes* larva. In the other of the two cells there were two larvae of unknown instars, but their identities could not be verified since the ethanol-filled glass vial in which they were stored broke during transport and its contents were destroyed. Two cells contained single fusiform larvae of unknown instars, both of which were barcoded (sample IDs: CMNTO_042, CMNTO_044) and thereby identified as *E. americanus* (see Fig. 2). Another four closed cells contained completely unexpected inhabitants: a live ground spider (Araneae: Gnaphosidae) in one and dead small ants in the three others. Presumably, these specimens entered the brood cells and became trapped when the female *Colletes* plugged each with a soil-filled cup. The collection data for the *Epeorus* first instar (presented below) are the same as for the other barcoded larvae. For the barcoded adult of *C. consors mesocopus*, the collection data are as follows: CANADA: **British Columbia:** Meadow Creek, clay pits (50.253,-116.984), 21.vi.2020, G.E. Hutchings (♀, CNC CMNTO_041).

Epeorus americanus (Cresson, 1878)

Fig. 3

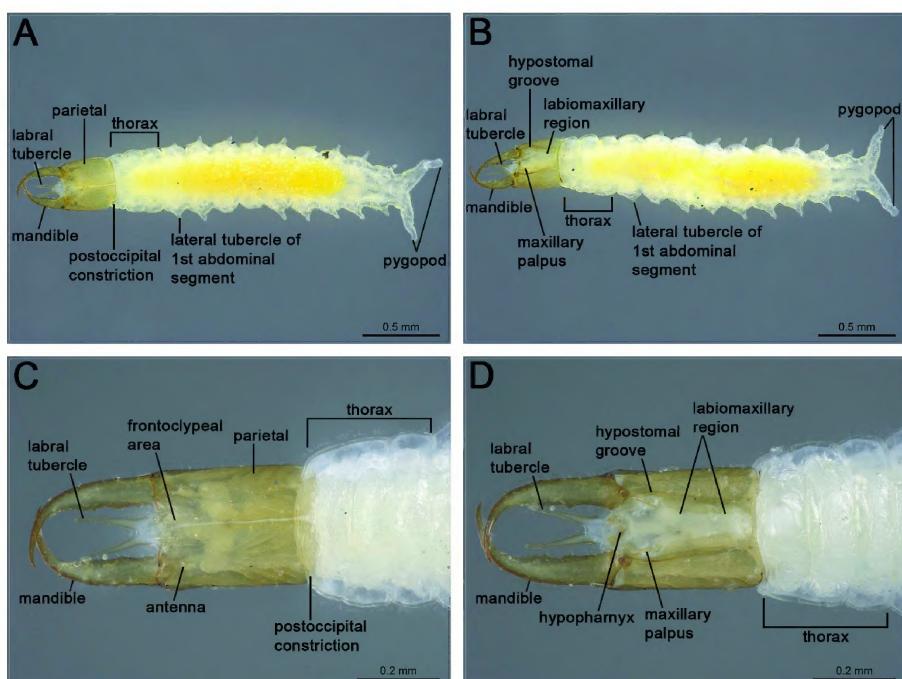


FIGURE 3. First instars of *Epeorus americanus*: (A) feeding larva (AMNH), habitus, dorsal view; (B) same feeding larva, habitus, ventral view; (C) newly eclosed larva (CNC), head and thorax, dorsal view; (D) same newly eclosed larva, head and thorax, ventral view.

Description

First instar

MEASUREMENTS. Medial length 1.73 mm; head medial length 0.38 mm; head basal width 0.28 mm; mandibular length 0.42 mm (newly eclosed larva; Fig. 3C, D) (N = 1).

HEAD. Mandible with prominent inner submedial swelling (slightly nearer the base than apex); inner margin of straight, basal two-thirds with series of minute irregular projections; margins of strongly incurved apical one-third smooth. Mandible in repose with anterior extent surpassing labral tubercle by about $\frac{1}{3}$ its length from base to tip. Head capsule in dorsal and ventral views with sides converging slightly anteriorly (apical width to basal width ratio = 0.9); in lateral view with frontoclypeal area projecting dorsally such that dorsal and ventral margins of head nearly parallel throughout length. Membranous V-shaped labiomaxillary region not strongly defined but differentiated from sclerotized parietal by slightly lighter coloration. Hypostomal grooves widely separated, such that in ventral view even shortest distance between them greater than distance between hypostomal groove and lateral margin of head.

BODY. Abdominal segments 1–9 each with pair of tapering lateral tubercles. Lateral tubercles triangular, with basal side somewhat greater than two subequal sides projecting laterally. Pygopod with posterolateral process (when fully extended) more than twice as long as longest lateral tubercle.

Material examined: CANADA: British Columbia: Meadow Creek (50.253,-116.984), 27.vi.2020, ex *Colletes cossors mesocopus* brood cell, G.E. Hutchings (1 larva, AMNH); same collection data as for preceding (1 larva, CNC); same collection data as for preceding (1 larva, barcoded, first author's collection CMNTO_040); same collection data as for preceding, except ex *Colletes cossors mesocopus* brood cell containing C-shaped *Colletes* larva (1 larva, PCYU).

Discussion

Through a combination of field observations, nest excavations, and the use of DNA barcoding to identify bee larvae recovered from unearthed *Colletes* brood cells, the first instar of *E. americanus* was identified and the species' association with *C. cossors mesocopus* confirmed in the present study. Whether or not *C. cossors mesocopus* is the only host of *E. americanus* remains to be seen, and some species of *Epeorus* have been associated with multiple host species (Onufenko *et al.* 2019). However, that both *C. cossors* Cresson and *E. americanus* should exhibit such similar distributions suggests that the former is the primary if not the only host of the latter (for comprehensive locality records, see Stephen 1954; Onufenko 2018; Ascher and Pickering 2020).

Few studies have described in detail the behavior of *Epeorus* females when searching for host nests. Whereas a detailed account by Graenicher (1906) of the behavior of a species of *Epeorus*, *E. minimus* (Robertson) (as *Argyroselenis minima*), suggests that

Epeolus females are clandestine, females of *E. americanus* at the Meadow Creek nest site readily explored host nest entrances and often entered them without pause. Our report of *E. americanus* females being repelled at nest entrances by host *Colletes* females is consistent with observations of another species pair by Bogusch (2003), who witnessed a female of *Colletes similis* Schenck forcing a female of *E. variegatus* (Linnaeus) away from its nest at a site in Buzice, Czech Republic. Since oviposition was not directly observed, it was not possible to distinguish between explorational and egg-laying visits by *E. americanus* females to *Colletes* nests. However, observations of another species, *E. pusillus*, by Rozen and Favreau (1968) suggest that the process of entering the nest, oviposition, and reemergence takes less than a minute.

The discovery of an *Epeolus* first instar in a cell with a larger *Colletes* larva was unexpected, given the homicidal nature of the former, as was the discovery of another cell with two larvae of unknown instars. These findings suggest that *Epeolus* larvae, within their short time as first instars, are perhaps not always successful in eliminating the competition within the nests of their hosts.

Nests of the host *C. consors mesocopus* exhibit similarities with those of other *Colletes*. Herein, it is reported that the species lines its brood cells with more than a single layer of lining, which is consistent with observations of other *Colletes* (e.g. *C. ciliatoides* Stephen and *C. compactus compactus* Cresson) (Torchio 1965; Rozen and Favreau 1968). Whereas females of certain species of *Colletes* are known to construct brood cells in linear series (e.g. of two to four cells in *C. hederae* Schmidt and Westrich and two to eleven cells in *C. kincaidii* Cockerell) (Torchio *et al.* 1988; Bischoff *et al.* 2005), the unearthed brood cells of *C. consors mesocopus* appeared singly at the end of a tunnel. This construction is consistent with that observed in many other *Colletes*, including for example *C. ciliatoides*, *C. compactus compactus*, *C. inaequalis* Say, *C. thoracicus* Smith, and *C. validus* Cresson (Torchio 1965; Rozen and Favreau 1968; Batra 1980; Almeida 2008). Brood cells of *C. consors mesocopus*, like those of *C. ciliatoides*, are almost entirely filled with provisions (Torchio 1965). In some *Colletes*, however, including *C. compactus compactus*, *C. halophilus* Verhoeff, *C. inaequalis*, *C. thoracicus*, and *C. validus*, which are larger bees that construct larger cells, the provisions occupy only the distal region of the cell (Rozen and Favreau 1968; Batra 1980; Almeida 2008; Rooijakkers and Sommeijer 2009; Sommeijer *et al.* 2012). The brood cells of *C. consors mesocopus* were quite close to the surface, with an observed maximum depth of around 7 cm. Some species construct much deeper nests. A nest of *Colletes stepheni* Timberlake, for example, a large species that is restricted to sand dunes, has been found about 98 cm below the dune surface, with the brood cell appearing at the end of a meandering tunnel that was approximately 140 cm in length (Hurd and Powell 1958).

The present article includes only the fourth description of the first instar of a species of *Epeolus* from North America and perhaps anywhere. The morphology of the first instar of *E. americanus* most closely matches the description of that of *E. compactus* among the species of *Epeolus* for which immatures have been described. At this time, the first instars of the two species cannot be distinguished from one another morphologically. In Rozen's (1989) key to species of *Triepeolus* Robertson (Hymenoptera: Apidae) and *Epeolus* based on first instars, the larva of *E. americanus* keys to *E. compactus*. In both species, the abdomen has nine pairs of tapering lateral tubercles, which are also present in several

species of the related genus *Triepelous* but absent in *E. ilicis* and *E. pusillus* (Rozen 1989). Curiously, *E. americanus* and *E. compactus* both belong to a clade informally referred to as the “Argyroselenis group” whereas *E. ilicis* and *E. pusillus* both belong to a clade informally referred to as the “Pyrrhomelecta group” (Onufreko *et al.* 2019). More taxon sampling from both species groups is required, however, to determine whether this difference between the two species pairs is evolutionarily linked. Searches for more *Epeolus* larvae should also be conducted to establish additional host-cleptoparasite associations, since hosts are still unknown for most species of *Epeolus*.

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THE EUROPEAN FIRE ANT, *MYRMICA RUBRA* (LINNAEUS) (HYMENOPTERA: FORMICIDAE), IN THE CREDIT RIVER WATERSHED

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Abstract

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The European fire ant, *Myrmica rubra* (Linneaus) (Hymenoptera: Formicidae), is an invasive species in Canada and the United States. *Myrmica rubra* has proven to be very hard to control once it has invaded an area, and preventing further spread of the species is important for moderating its negative effects. There are limited studies of this species in Ontario. We used pitfall traps to sample *M. rubra* at ten wetlands in the Credit River watershed and explored the relationships between three factors—disturbance, wetness, and proportion of urban land use surrounding the wetlands—and *M. rubra* incidence and abundance. Three of the ten sites had a very high abundance (>30,000 ants) of *M. rubra*. The proportion of surrounding urban cover and disturbance had strong positive relationships with both the incidence and abundance of *M. rubra*. Ecological and land use data predicting *M. rubra* presence and abundance can assist in identifying sites that may be vulnerable to future invasions. We recommend incorporating ant monitoring into existing long-term monitoring efforts in southern Ontario.

Introduction

The European fire ant, *Myrmica rubra* (Linneaus), is an invasive species in North America. First detected in Massachusetts in the early 1900s, it is now found across the northeastern United States and southeastern Canada, as well as in Washington State and British Columbia (Groden *et al.* 2005; Wetterer and Radchenko 2011; Naumann and Higgins 2015; Chen and Adams 2018). Multiple impacts of the European fire ant in North America have been documented, including on reducing native arthropod biodiversity (Naumann and Higgins 2015; Verble-Pearson and Pearson 2016; Goodman 2018) and slowing growth rates in herring gull (*Larus argentatus* Pontoppidan) chicks (DeFisher and Bonter 2013). Field

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and lab studies have also shown that *M. rubra* can increase the abundance of plant-feeding homopterans (McPhee *et al.* 2012), modify seed dispersal processes (Prior *et al.* 2015; Gammans *et al.* 2018), competitively displace native ants (Garnas *et al.* 2014), and disrupt pollination (Cembrowski *et al.* 2014). Their ability to form supercolonies, wherein ants from different nests separated by a certain distance are not aggressive towards each other, provides them with a competitive advantage and allows them to build up very high densities within infested areas (Garnas *et al.* 2007; Naumann *et al.* 2017; Chen *et al.* 2018; Warren *et al.* 2019b).

Although it has been present in Ontario since at least 1975 (Groden *et al.* 2005), the distribution and ecology of *M. rubra* in the province has only begun to be studied. Khan (2018) collected reports of the species from published works, as well an online survey, and documented its presence across southern Ontario from Oshawa (Durham Region) in the east to Guelph (Wellington County) in the west. All field studies of the European fire ant in Ontario have focused on the Greater Toronto Area and have included investigations of its actual and potential interactions with native and invasive species (Rudmik 2011; Prior *et al.* 2015; Meadley Dunphy *et al.* 2016; Gupta *et al.* 2017; Khan 2018), a description of its population genetics in Toronto (Meadley Dunphy 2016), and a study of the ecological variables related to its presence at conservation areas (Ito 2014).

Ito (2014) found that soil moisture, soil temperature, and elevation were all significant predictors of *M. rubra* presence at 15 conservation areas in the Greater Toronto Area. However, these results may have confounded elevation with habitat type; Ito's (2014) study sites with higher elevations—and few to no *M. rubra*—were upland habitats such as meadows and dry forests. Previous research has shown soil moisture to be a key factor in determining *M. rubra* presence in New England (Groden *et al.* 2005; Chen and Adams 2018), Ontario (Rudmik 2011), and New York (Warren *et al.* 2019a), as well as for other invasive ant species (Tschinkel 1988; Holway 2005; LeBrun *et al.* 2012). More broadly, the distribution and abundance of invasive ant species is often related to the amount of anthropogenic influence; in their review of the ecology of ant invasions, Holway *et al.* (2002) report that disturbed habitats are more susceptible to invasion by ants than natural habitats.

In combination with their ability to form supercolonies, the European fire ant's aggressive nature and painful sting makes it a concern for park managers and municipalities where it is established (Groden *et al.* 2005; Wetterer and Radchenko 2011). Control options for *M. rubra* are limited; pesticides can temporarily reduce local populations but have not been effective at completely removing them (e.g. Warren *et al.* 2019a), and research on biological control is in its early stages (Simmons *et al.* 2015). Increased knowledge of the site characteristics associated with the presence and abundance of *M. rubra* in Ontario could assist in predicting its current and future distribution, thereby helping natural resource managers and landowners prevent establishment or locate and eradicate early infestations. In this study, we investigated how soil moisture, disturbance, and degree of urban surroundings were related to *M. rubra* abundance at ten wetland sites. Due to the small sample size in our study and the general paucity of information on *M. rubra* in Ontario, we have chosen to use the data collected in our study in hypothesis generation as opposed to using it to test a particular hypothesis. Our aim is that the results from this work will be used to direct continuing research on this invasive species.

Materials and methods

Study sites

European fire ants were sampled at ten wetlands within the Credit River watershed in southern Ontario (Fig. 1; Table 1). The sites were selected from a set of wetlands regularly monitored by Credit Valley Conservation (CVC) based on 1) accessibility; 2) previous anecdotal fire ant observations, ranging from none to infested; and, 3) location in the watershed, with an aim to sample sites throughout the entire jurisdiction. The sites that met these criteria included two types of wetlands—swamps and marshes—as classified by the Canadian Wetlands Classification System. Swamps are dominated by trees or tall shrubs with the water table below a major area of the ground surface whereas marshes are wetter, with shallow levels of standing or slow-moving water, with vegetation primarily composed of aquatic macrophytes (National Wetlands Working Group 1997).

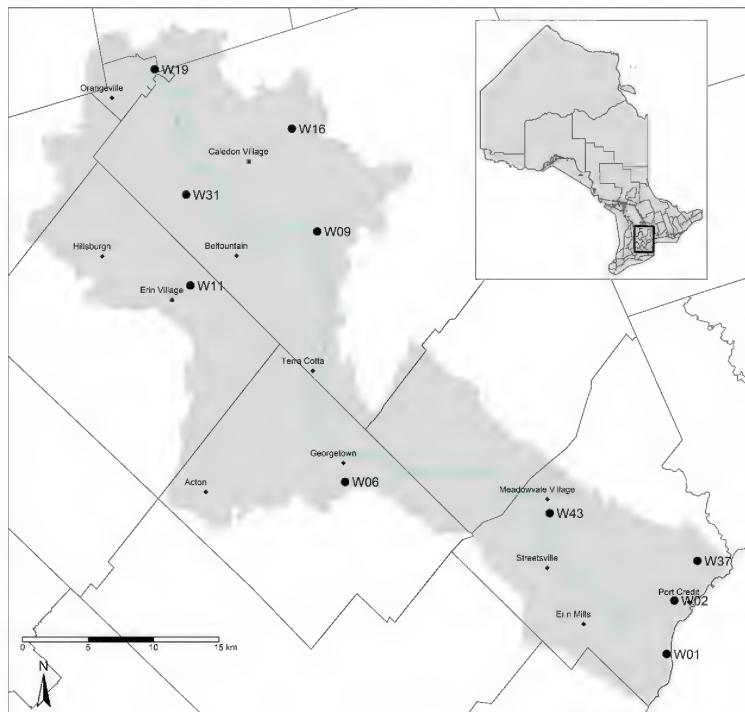


FIGURE 1. Wetlands within the Credit River watershed, Ontario, sampled for *M. rubra* in 2016. The watershed boundary is indicated by gray shading, township boundaries by the thin black lines, and the main Credit River by the blue line. Black circles represent the location of sites (see Table 1 for corresponding site names).

TABLE 1. Characteristics of ten wetland sites sampled for *M. rubra* in 2016: site number and name; geographic coordinates (given as decimal degrees); wetland community type; and the pre-study anecdotal estimates of *M. rubra* abundance.

Site no.	Site name	Latitude	Longitude	Wetland community type	<i>M. rubra</i> estimates pre-study
W01	Rattray Marsh	43.517	-79.607	marsh	many
W02	Credit River Marshes	46.554	-79.599	marsh	some
W37	Cawthra	46.581	-79.576	swamp	none
W43	Meadowvale South	43.615	-79.715	marsh	some
W06	Hungry Hollow	43.639	-79.909	marsh	many
W11	Erin Pine	43.775	-80.053	marsh/swamp	some
W09	Ken Whillans	43.812	-79.932	thicket/swamp	some
W31	Alton	43.838	-80.056	swamp	none
W16	Starr	43.882	-79.955	marsh	none
W19	Melville	43.925	-80.085	marsh	none

CVC maintains permanent transect lines at each wetland site, to facilitate vegetation surveys as part of a long-term watershed monitoring program (CVC 2013a). Each transect is 40 m long; 1 × 1 m vegetation survey plots were set 5 m out on both sides of the center line at every 10 m along the transect, for a total of 10 vegetation survey plots per transect.

Insect collections

Ants were sampled between 14 and 22 June 2016 using pitfall traps. Ten pitfall traps were placed along the permanent CVC transect; one on either side of the transect line at each 10 m mark, as for the vegetation survey plots described above, but set an additional 10 m from the center of the transect line. Traps (650 g) were filled halfway with diluted propylene glycol, to which a small amount of dish soap was added as a surfactant. Traps were placed within a hand-dug hole so that the upper lip was flush with the ground, and then covered with a raised corrugated plastic roof to prevent rainwater from entering. A chicken-wire fence surrounded each trap to prevent the trapping of non-target organisms, particularly vertebrates. Following Naumann and Higgins (2015), traps were deployed for seven days, after which their contents were strained, rinsed with water, and stored in 95% ethanol prior to processing.

Ant identification and abundance

For samples containing low or intermediate numbers of ants (<1,000), all specimens were counted and identified, using Ellison *et al.* (2012). Samples from three sites that contained very large numbers of *M. rubra* individuals per trap (i.e. >1,000) were scanned for ants of other species, dried in an oven at 65 °C for 24 hours, and weighed to assess the approximate total number of *M. rubra*. A selection of ants from each of the larger samples were removed and identified to ensure that the sample was entirely composed of *M. rubra*. To determine average ant mass, five random samples of 20 ants were removed

from each sample without out replacement and weighed using an 100G/0.001G B1003T Electronic Balance Laboratory scale. The average was consistent among the samples and used to determine the total number of ants in each weighed sample. While the relationship between biomass and abundance can vary from weak to strong in terrestrial arthropods, it is generally strong for samples collected using pitfall traps and restricted within taxonomic groups (e.g. families, species), as was the case in our study (Saint-Germain *et al.* 2007).

Environmental variables

As direct measures of site wetness and disturbance were not available, we chose to use proxies for these variables based on the composition of the vegetation community at the survey sites. As one component of CVC's wetland monitoring protocols, all vascular plants within the ground vegetation and regeneration layers of the monitoring plots are identified to species (CVC 2013a). Each plant species in the database has an associated wetness score in the Floristic Quality Assessment System for Southern Ontario; the value is between -5 and +5, with -5 being an obligate wetland species and +5 an obligate upland species (Oldham *et al.* 1995; CVC 2010). A mean wetness score can be calculated for a site from the individual scores for each plant species present. This provides a representative value for the wetness of the habitat, with lower and negative numbers representing wetter sites. Values for the plant-based wetness index have been shown to have strong correlations with directly measured values of soil moisture at wetlands in southern Ontario (Francis *et al.* 2000).

Similarly, we chose to use the proportion of non-native plant species present in the survey plots at each wetland as a measure of anthropogenic disturbance. Our hypothesis was that sites with a higher proportion of non-native species were more likely to have higher levels of anthropogenic disturbance. This is supported by research showing strong correlations between the proportion of non-native plant species at a site and disturbance factors such as agriculture, road building, recreation, as well as housing and industrial development (e.g. Chytrý *et al.* 2008; McKinney 2008; LaPaix *et al.* 2009). Data from the last two sample years at each site were used to calculate habitat wetness and disturbance (2013–2016; Table 2). The average over the two years was used as the value for each site.

Finally, the proportion of urban cover in the landscape surrounding each site was taken from a CVC landscape analysis (CVC 2013b). In this analysis, ArcGIS was used to categorize land use in the Credit River watershed as either natural, urban, or agricultural. The amount of each land use type was calculated for an area of 2,000 m surrounding a 30 m buffer around the centre of each monitoring site (CVC 2013b). The total area of urban use was summed and divided by the total land area to determine the proportion of urban land cover surrounding each site.

Data exploration

In the context of hypothesis generation, we used scatterplots to visually assess the relationships between mean wetness, the proportion of non-native plant species, and the proportion of surrounding urban land use with two ant response variables—the proportion of pitfall traps occupied by *M. rubra* (i.e. trap-based incidence) and the log-transformed, standardized abundance of *M. rubra* and at each site.

We focused on incidence as our primary measure of *M. rubra* at each site, acknowledging that the number of individual worker ants collected in each pitfall trap was

TABLE 2. Sampled abundance of *M. rubra* and habitat characteristics of ten wetland sites sampled in 2016; total abundance of *M. rubra* collected (Abd.); the number of pitfall traps in which *M. rubra* was collected; the total number of pitfall traps used for collections; the log-transformed, trap-standardized abundance (Log abd.); mean wetness index; proportion of non-native plant species present in the ground vegetation; proportion of the surrounding land use made up of urban habitats; and the years in which vegetation sampling was conducted and from which the mean wetness index and proportion of non-native plant species were calculated.

Site no.	Site name	Abd.	Traps w. <i>M. rubra</i>	Total traps ^b	Log abdd.	Wetness index ^c	Non-native species	Urban cover	Vegetation sampling
W01	Rattray Marsh	56,962 ^a	10	10	3.76	-3.49	0.29	0.45	2015, 2016
W02	Credit River Marshes	30,812 ^a	8	8	3.59	-2.41	0.31	0.68	2014, 2016
W37	Cawthra	517	6	10	1.72	-0.21	0.13	0.80	2015, 2016
W43	Meadowvale South	25	8	10	0.54	-1.89	0.19	0.55	2014, 2016
W06	Hungry Hollow	103,615 ^a	10	10	4.02	-3.39	0.21	0.56	2013, 2015
W11	Erin Pine Estates	13	3	10	0.36	-3.61	0.06	0.32	2014, 2016
W09	Ken Whillans	0	0	9	0.00	-2.24	0.29	0.13	2015, 2016
W31	Alton	5	2	10	0.18	-2.61	0.07	0.07	2014, 2016
W16	Starr	0	0	9	0.00	-3.60	0.10	0.07	2015, 2016
W19	Melville	47	7	10	0.76	-3.80	0.18	0.46	2013, 2015

^aTotal abundance of *M. rubra* at these sites was not counted directly but rather estimated using the mass of dried samples (see Methods for details).

^bSome sites had fewer than 10 sample traps due to an inability to place traps in areas with standing water or due to traps being disturbed.

likely to be highly dependent on its proximity to one or more colonies and may overestimate the true number of colonies at the site (Longino 2000; Gotelli *et al.* 2011). Nonetheless, we have also included the total abundance of *M. rubra* at each site as a response variable as it better reflected the differences among sites. For example, we considered that the difference in *M. rubra* populations between Meadowvale South (25 individual ants, 8/10 occupied traps) and Credit River Marshes (estimated 30,812 ants, 8/8 occupied traps) was larger than implied by the difference in incidence alone. Prior to plotting, the total number of *M. rubra* at each site was standardized for sample effort by dividing by the total number of the traps used at the site. Standardized ant data were further log-transformed to account for the large differences in *M. rubra* abundance among sites.

Results

Eight of the ten sites sampled had *M. rubra*; three were heavily infested (>30,000 ants collected), one had a relatively moderate presence (>500), and the remaining four sites had very low (<50) presence (Table 2). At Starr and Credit River Marshes, not all traps could be placed along the transect line due to high water levels. At Ken Whillans, one of the traps was overturned. Three of the sites with moderate to heavy *M. rubra* infestations (Rattray Marsh, Cawthra, and Credit River Marshes) were geographically very close together, while one (Hungry Hollow) was more isolated (Fig. 1).

Scatterplots of our data showed positive relationships between both the proportion of invasive plants and the proportion of surrounding urban land use with both ant incidence and ant abundance. By contrast, there was no relationships between mean wetness and both ant response variables indicated by horizontal trendlines (Fig. 2).

Discussion

In this study, we estimated the strength of the relationship between three environmental factors—wetness, proportion of non-native plants (as a measure of disturbance), and surrounding urban cover—and *M. rubra* incidence and abundance at wetlands throughout the Credit River watershed in southern Ontario. Both ant incidence and ant abundance increased with increasing disturbance as well as with an increasing amount of urban cover in the surrounding area. Below we discuss some potential explanations of our observations and suggest directions for additional research.

The positive relationship between the proportion of urban cover and *M. rubra* abundance is a new finding for this species. Like other invasive ants, the spread of *M. rubra* in North America is likely largely due to the movement of infested plants, soils, and other materials (Groden *et al.* 2005; Chen and Adams 2018). For this reason, urban settings and developed areas are often associated with higher abundance and dominance of invasive ant species. The distribution of the Argentine ant (*Linepithema humile* (Mayr)) has been shown to be influenced by human land use both globally (Roura-Pascual *et al.* 2011) and locally (Carpintero *et al.* 2004; Bolger 2007; Fitzgerald and Gordon 2012). The establishment of red imported fire ant (*Solenopsis invicta* Buren) colonies also increases

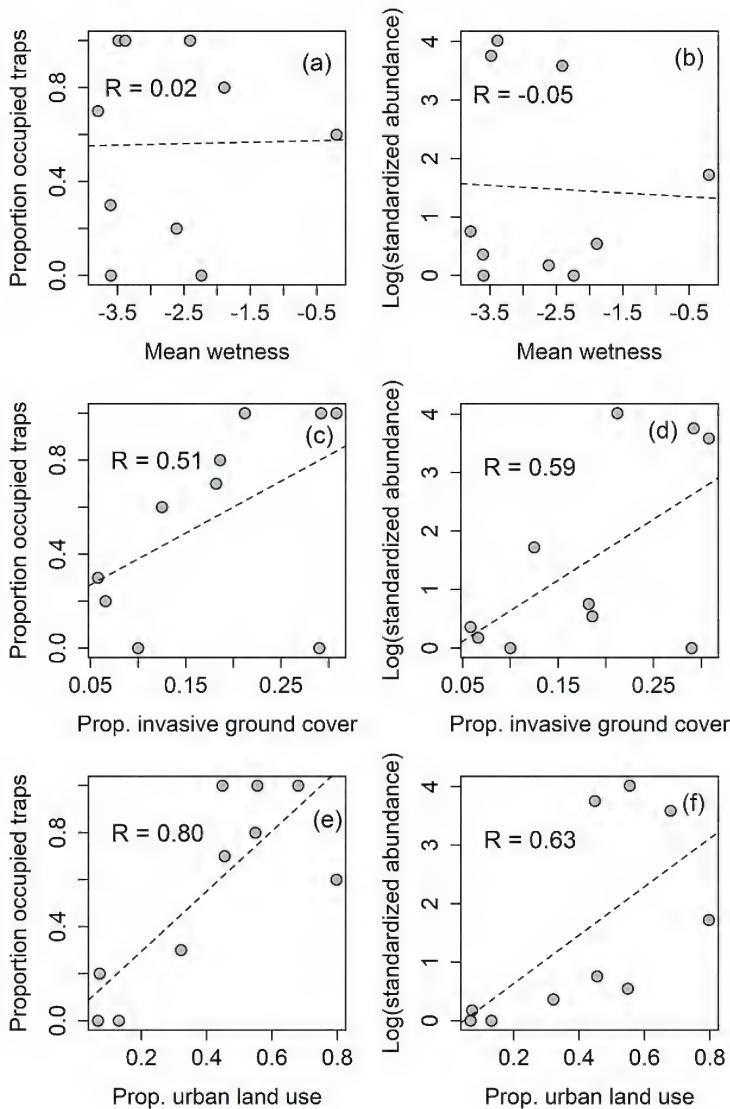


FIGURE 2. Scatterplots showing the relationships between mean wetness (a and b), proportion of non-native plants in the ground vegetation (c and d), and proportion of urban land use in the surrounding 2,000 m (e and f) with the trap-based incidence of *M. rubra* (a, c, e) as well as the log-transformed standardized abundance of *M. rubra* (b, d, f) at ten wetlands in the Credit River watershed, Ontario. The correlation coefficient Pearson's R is shown on each scatterplot.

near human dwellings (Forys *et al.* 2002). The relationship between urbanization and invasive ants extends beyond these two well-known species; Lessard and Buddle (2005) found that the relative abundance of the introduced pavement ant, *Tetramorium caespitum* (Linnaeus) increased along a transect from natural to urban sites in Quebec.

Anthropogenic disturbance, in the form of mowing, plowing, and other soil excavations, has been found to be related to increased prevalence and densities of both the Argentine ant (King and Tschinkel 2008) and the red imported fire ant (Tschinkel 1988; LeBrun *et al.* 2012). It can be difficult to separate the effects of urbanization from those of anthropogenic disturbance; the two are generally related since humans disturb areas that are close to developed areas, either as a result of development itself or because of proximity to development. In our study, Pearson's R for the variables we used to represent disturbance and urbanization (proportion of non-native plant species present in the ground vegetation and the proportion of the surrounding land use made up of urban habitats, respectively) was estimated to be 0.32. Although this is not an insignificant relationship, we did not feel that it was strong enough to make investigating the relationships of both environmental variables with ant incidence and abundance redundant. It is possible that we would have found a stronger relationship between anthropogenic disturbance and *M. rubra* abundance if we had used a direct measure of disturbance rather than a measure inferred from the proportion of invasive plants. However, finding a variable to provide a direct measure of disturbance can be challenging and using existing monitoring data was parsimonious. In addition, we note that in at least one of the studies cited above, the disturbed areas with high ant abundance also had an increased prevalence of weedy plant species (Tschinkel 1988), lending support to our use of the proportion of invasive species as a proxy for disturbance.

Habitat wetness showed no relationship with *M. rubra* incidence and abundance in our study. We expected that wetness would show a positive relationship with *M. rubra* incidence and abundance based on literature that suggests it is an important factor in the ecology of invasive ant species, including the European fire ant (Gorden *et al.* 2005; Rudmik 2011; Ito 2014; Chen and Adams 2018; Warren *et al.* 2019a). It is possible that the use of direct soil moisture measurements instead of a vegetation-based proxy may have led to stronger association between wetness and *M. rubra* abundance, as has been seen in other studies. However, we must also consider that the small range of variation in wetness in our study may explain the lack of a directional relationship. All of our study sites were wetlands, which seem to be the preferred habitat for the European fire ant. In a large survey of southern New England, Chen and Adams (2018) found that the species was most prevalent in freshwater marshes and wet meadows as well as wetter forests, riparian habitats, and forested wetlands while dry forests, old fields, and mown grass were rarely used.

In terms of identifying other factors affecting *M. rubra* distribution, we suggest that future research might benefit from examining both the landscape scale and microhabitat scale. Although much of the spread of *M. rubra* is due to human-assisted movement, colonies also expand naturally into adjoining areas (Groden *et al.* 2005; Chen and Adams 2018). Given that many *M. rubra* populations are in wetlands in riparian areas, their spread may be facilitated by the connectivity of suitable habitat in these natural corridors as well as the movement of individuals or even nests by water as with other invasive species (e.g. Hood and Naiman 2000). A landscape assessment of suitable habitat combined with connectivity

to existing populations may help to locate new or potential *M. rubra* nesting infestations. At the microhabitat scale, recent research in the United States has described the general habitat types and abiotic conditions most favoured by *M. rubra* (Chen and Adams 2018; Warren *et al.* 2019a). Gathering more data with respect to the particular soil types and plant species associated with *M. rubra* presence and abundance would assist in understanding the potential distribution, impact and management of this species.

Our study built upon existing monitoring efforts of wetland habitats in the Credit River watershed. We suggest incorporating an ant monitoring program, which could be linked to other biotic wetland monitoring programs such as CVC's anuran monitoring (CVC 2010), permitting an analysis of the impact of *M. rubra* on amphibian biodiversity. Wetlands provide numerous ecosystem services, including critical habitat for rare and threatened species. The majority of wetlands in southern Ontario have been converted to agricultural or urban uses over the past 200 years (Ducks Unlimited Canada 2010), and the remaining wetlands face numerous threats including ongoing conversion, climate change, and invasive species. Our findings suggest that wetlands within urban landscapes are more likely to have high *M. rubra* abundances, posing another significant threat to the biodiversity in these sensitive communities. Land managers can focus efforts on informing the public of the presence of *M. rubra* at known locations, recommending that users of urban wetlands be on the lookout for new infestations, and restricting movement of soil or other biotic material to reduce the potential spread of this species.

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NOVEL MUTUALISTIC INTERACTION IN INTRODUCED *POLYOMMATUS ICARUS* (ROTTEMBURG) LARVAE IN QUEBEC

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Abstract

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The European common blue *Polyommatus icarus* (Rottemburg) (Lepidoptera: Lycaenidae) was introduced to Quebec in 2005 and has since become successfully established within the province. Throughout its native range, the caterpillar forms a facultative association with ants for protection from predators and parasitoids. Here, we document a novel myrmecophilous interaction between *P. icarus* and a native ant species, *Lasius neoniger* Emery (Hymenoptera: Formicidae). The only native lycaenid observed in the same habitats, *Glaucopsyche lygdamus* (Doubleday), also interacted with *L. neoniger* as a caterpillar, but displayed a different phenology from *P. icarus*. These findings suggest that myrmecophilous relationships, like other mutualisms, can readily emerge between novel partners under changed community contexts.

Introduction

Mutualist species from different geographic ranges that come into contact may form novel relationships via ecological fitting. This is more likely to occur if both form associations with species with similar traits in their respective native distributions (Traveset and Richardson 2014). Lycaenidae is a family of Lepidoptera represented by species that often maintain mutualistic or parasitic relationships with ants, an interaction known as myrmecophily (Pierce and Mead 1981; Fiedler and Hölldobler 1992). Symbiotic relationships are more common during the larval or pupal phase and may be obligatory or optional (Jordano and Thomas 1992). Myrmecophily has proven to be the insect-insect relationship with the greatest ecological impact in these groups, directly influencing the relationships between lepidopteran larvae and both their host plants (Fiedler 1990) and

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other insects (Pierce and Mead 1981), as well as even inducing a selective pressure for the development of specialized bodies (Fiedler 1990; Pierce *et al.* 2002). In this type of interaction, the larvae generally have morphological adaptations (dorsal nectary organ) whose function is to appease ants and attract them by offering a nutritious reward (Malicky, 1970). In return, the ants protect the larvae against natural enemies (Pierce *et al.* 2002; Kaminski and Rodrigues 2011; Fiedler 2012).

The European common blue *Polyommatus icarus* (Rottemburg) (Lepidoptera: Lycaenidae) was first observed in the Montreal region (Quebec) in 2005 (Layberry and Jones 2008) and has since become prolific (eButterfly 2020). The host plants of *P. icarus*, ruderal herbs in the family Fabaceae, were also introduced in North America generations ago, and are now common urban plants in Montreal (Marie-Victorin *et al.* 2002). *Polyommatus icarus* belongs to the family that includes the blue, copper, and hairstreak butterflies, all generally small species that typically inhabit open habitats. They are represented in most parts of the world. *Polyommatus icarus* is one of the most widespread species in its native range, comprising most of Europe and parts of Western Asia. The species is multivoltine, having two to four generations per year depending on the local climate. Like most Lycaenidae of the subfamily Polyommatinae, *P. icarus* forms myrmecophilous associations in the late larval instars, as described above.

The adaptive advantage of myrmecophily in lycaenid caterpillars in North America is the same as in European species; most are attacked by parasitoids, such as Braconidae (Hymenoptera) and Tachinidae (Diptera) (Pierce and Mead 1981; Fiedler *et al.* 1992). Exclusion of ants results in higher parasitization rates in larvae, which no longer benefit from ant protection (Pierce and Mead 1981). The secretions the larvae produce to maintain ant presence are rich in carbohydrates (Daniels *et al.* 2005). Since *P. icarus* is facultatively myrmecophilous, we investigated whether ant-larva interactions were maintained in the introduced population in the area of Montreal and if so, which ant species were involved.

Materials and methods

Study sites

Two distinct sites on the Island of Montreal containing introduced Fabaceae were surveyed for lycaenid larvae from June to August 2019. Adult *P. icarus* observations at both these sites are documented in the e-Butterfly citizen science database (eButterfly 2020). The first site is an unmowed 2.8 ha old-field next to the visitor's parking area of McGill University's Morgan Arboretum (45°25'47.7"N; 73°56'34.3"W). The site is an open habitat with mostly tall grass vegetation. The second site is the City Farm School on Concordia's Loyola campus (45°27'34.0"N; 73°38'33.0"W), which contains a permaculture garden (150 m²) in a matrix of urban parkland. The permaculture is used to grow ornamental flowers, including nectariferous species that attract pollinators.

Data collection

Butterflies were identified in the field when at rest. *Polyommatus icarus* is distinguishable from native lycaenid species by the presence of distinct orange spots on the underside of both pairs of wings in both males and females. Collected ant specimens were

identified in the lab with the help of a dissecting microscope and a field guide to ants of New England (Ellison *et al.* 2012).

To test for temporal niche overlap in these two co-occurring lycaenid species, citizen-science data from the e-Butterfly database was queried for observations of adult *P. icarus* and *Glaucopsyche lygdamus* (Doubleday) (Lycaenidae) between 2014 and 2019 in a quadrat centered on Montreal (46°N; 73°W – 46°N; 76°W by 44°N; 73°W – 44°N; 76°W).

Results

Polyommatus icarus early instar (<5 mm) caterpillars ($N = 10$) were observed in the City Farm School on 29 August 2019, feeding on *Medicago lupulina* Linnaeus (Fabaceae) and *Lotus corniculatus* Linnaeus (Fabaceae), both introduced from Europe. Two of the ten larvae were tended by a native ant, *Lasius neoniger* Emery (Hymenoptera: Formicidae) on *M. lupulina* (Fig. 1A). The ant's behavior corresponded to the description of groping by Malicky (1970), whereby the ants touch the larva with their antennae at an obtuse angle when the ants are not excited. The ants were also tending aphids present on the host plant. Late instar (>5 mm) *G. lygdamus* ($N = 6$) larvae were observed on 3 July 2019 at the Morgan arboretum feeding on *Vicia cracca* Linnaeus (Fabaceae), a plant species also introduced from Europe. The caterpillars were also tended by *L. neoniger* ants (Fig. 1B).

During subsequent fieldwork done in July 2020, five more larvae were spotted in Parc de la Cité in Longueuil feeding on white sweet clover (*Melilotus albus* Medik. (Fabaceae)) (45°29'21.8"N; 73°24'27.6"W), three of them tended by *Lasius neoniger* (Dexheimer 2021). Ant specimens collected in the summer of 2020 have been submitted to the Ouellet-Robert collection at Montreal University (*P. icarus* collection numbers: 56609, 56610, 56611, 56612; *L. neoniger* collection number: 56608). Adult *P. icarus* were also observed near the Arboretum in highway margins outside the park, and no other lycaenids, adults or larvae, were observed at either site.

The flight periods of both butterfly species showed significantly different phenological patterns (Fig. 2, Pearson's chi-squared test, $\chi^2_5 = 209.26$, $P < 0.0001$). Indeed, *G. lygdamus* is known to be univoltine and to hibernate as a pupa (ITIS 2020), whereas *P. icarus* is multivoltine, exhibiting two to four generations per year in different parts of its native range, and overwintering as a larva (Eeles 2019).

Discussion

Lasius neoniger is ecologically dominant in urban habitats in Montreal (Lessard and Buddle 2005). It is thus both abundant in habitats occupied by *P. icarus* larvae and an opportunistic species that does well in novel community contexts. The discovery of this novel association adds the first North American ant to the list of species known to interact with *P. icarus* (Handfield 1999). This supports previous research showing that facultatively myrmecophilous lycaenids can associate with a broader range of ant species compared with parasitic lycaenids (Fiedler 2001; Pierce *et al.* 2002). Parasitic lycaenids associate more

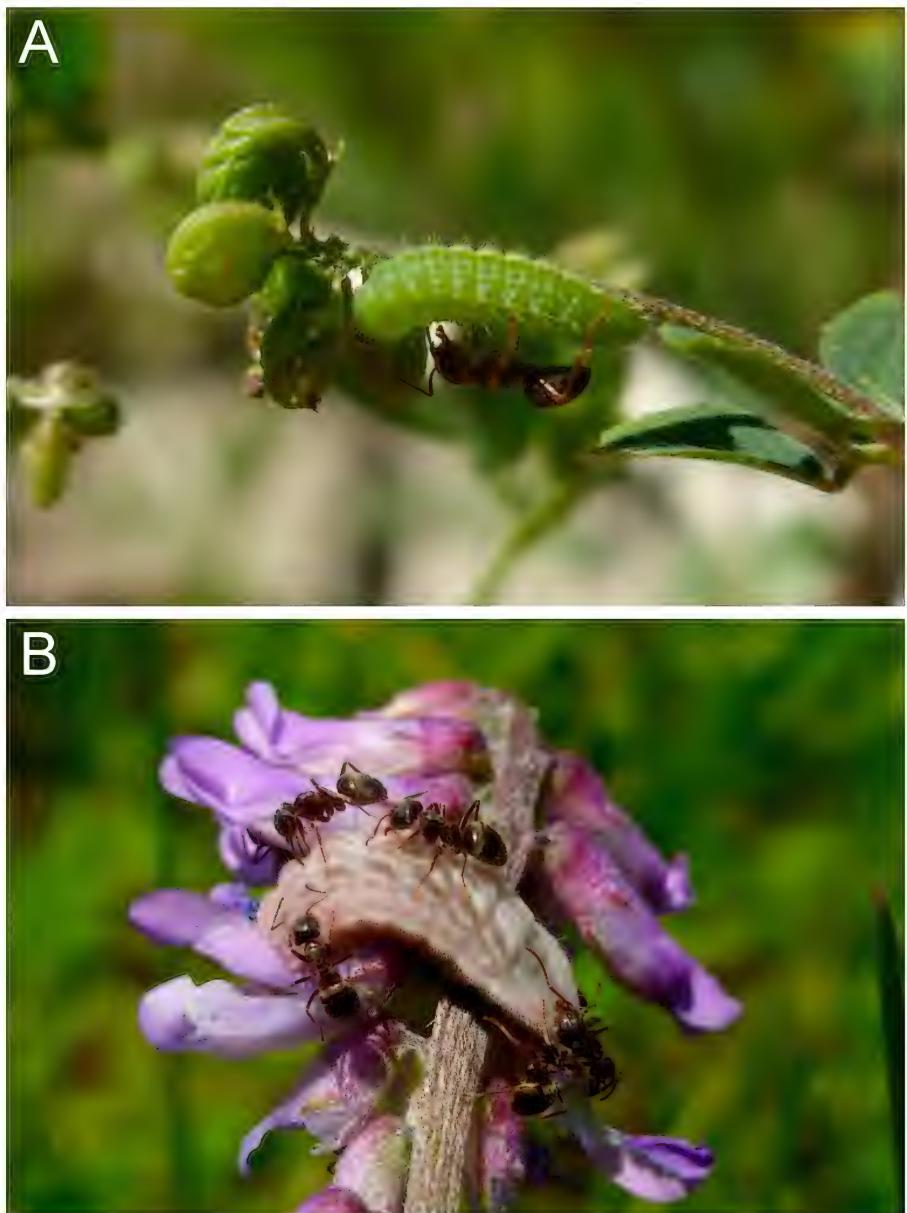


FIGURE 1. (A) *Polyommatus icarus* caterpillar, tended by *Lasius neoniger* on black medick (*M. lupulina*). (B) *Glaucopsyche lygdamus* caterpillar tended by *L. neoniger* ants on tufted vetch (*Vicia cracca*). Photos: Eric Dexheimer.

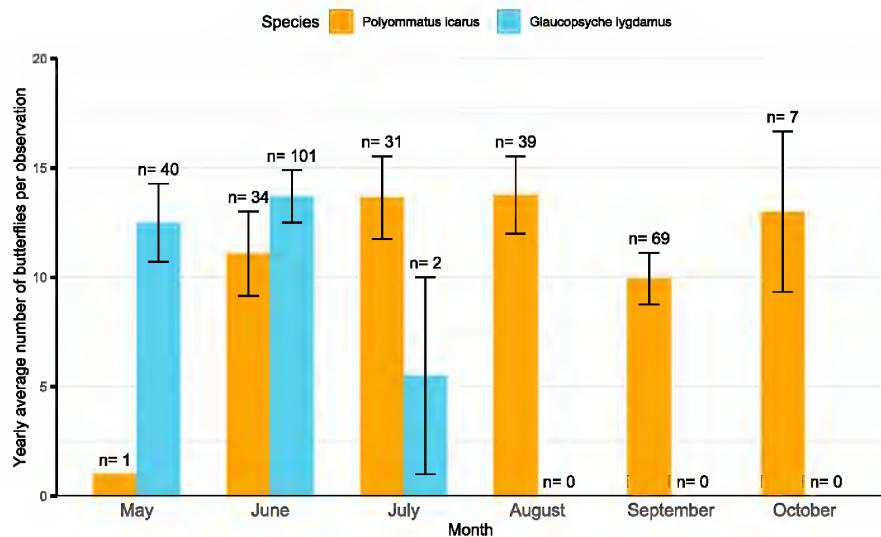


FIGURE 2. Average monthly number of observations between 2017 and 2019 (standard error bars) of *Glaucopsyche lygdamus* (blue bars, n = 143 observations) and *Polyommatus icarus* (orange bars, n = 181 observations) adults in the Montreal, Quebec region. Data from e-Butterfly (2020). Only one observation of *P. icarus* has been submitted in May throughout the years, and thus error bars could not be calculated for this month. The letter ‘n’ indicates the cumulated number of submitted observations from 2017 and 2019, the years used to calculate the average values.

closely with ants in that they feed on ant larvae within their ant host’s nest, and associate with fewer ant species as a result of their specialized life cycle (Als *et al.* 2001; Dierks and Fischer, 2009).

This has led to further research in the role of host plant quality in secretion composition, attractiveness to ants, and the intensity of myrmecophilous relationships in lycaenids (Fiedler and Maschwitz, 1989; Fiedler, 1990, 1995, 1996; Burghardt and Fiedler, 1996; Fraser *et al.* 2001). Pierce and Elgar (1985) suggested that Lycaenidae preferred Fabaceae host plants due to their high concentration of nitrogen, but Fiedler (1995, 1996) found that only twenty percent of all obligate myrmecophilous lycaenids use them as hosts. This suggests Fabaceae are not vital to the larvae for maintaining ant presence through nutrient-rich secretions.

Lasius neoniger and *G. lygdamus* have been shown to partake in myrmecophily (Tables S1 and S2), and the latter has been consistently used as a study system as it is a common species (Pierce and Mead, 1981; Pierce and Easteal, 1986; Fraser *et al.* 2001). Thus, this ant species has evolutionary experience with at least one native lycaenid species

with similar anatomical traits to *P. icarus* (production of nutritive secretions from a dorsal nectary organ upon mechanical stimulation) but different phenologies. The different phenology of *P. icarus* and *G. lygdamus* suggest that competition between them will be limited and furthermore, that the *P. icarus* / *L. neoniger* interaction is truly novel.

Disruption of mutualisms is increasingly common under global change, as a consequence of species introductions but also global warming and habitat disturbance (Telfer *et al.* 2005; Memmott *et al.* 2007; Tylianakis *et al.* 2010; Morales *et al.* 2017; Rogers *et al.* 2017; Brambilla *et al.* 2020). Our results support the emerging generalization that species can establish mutualisms with novel partners (Traveset and Richardson, 2014). In this instance, the pre-adaptations of a myrmecophilous species allowed it to integrate a novel species in its interaction network.

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APPENDIX 1. Supplementary tables.

TABLE S1. Lycaenidae in Quebec known to form myrmecophilous associations (from: Canadian Biodiversity Information Facility website, 2019) (Handfield 1999).

Subfamily	Vernacular	Species	Tending ant species	Host plants
Polyommatinae	Spring azure	<i>Celastrina ladon</i> (Cramer)	<i>Formica subsericea</i> , <i>Camponotus nearcticus</i> , <i>Crematogaster lineolatus</i>	<i>Prunus</i> L. spp., <i>Vaccinium</i> L. spp., <i>Viburnum</i> L. spp.
	Summer azure	<i>Celastrina neglecta</i> (Edwards)	Unspecified	
	Cherry gall azure	<i>Celastrina</i> Tutt sp.		<i>Prunus</i> spp., <i>Viburnum</i> spp.
	Square-spotted blue	<i>Euphilotes battoides</i> (Behr)	Unspecified	<i>Eriogonum</i> Michx. spp.
	Rocky mountain dotted blue	<i>Euphilotes ancilla</i> (Barnes and McDunnough)	Unspecified	<i>Eriogonum</i> spp.
	Western tailed blue	<i>Everes amyntula</i> (Boisduval)	Unspecified	Fabaceae (<i>Lathyrus</i> L. spp.)
	Silvery blue	<i>Glauopsyche lygdamus</i> (Doubleday)	<i>Formica microgyna</i> (?), <i>F. subsericea</i> , <i>F. oreas comptula</i> , <i>Tapinoma sessile</i>	Fabaceae
	Reakirt's blue	<i>Hemiargus isola</i> (Reakirt)	Unspecified	<i>Prosopis</i> L. spp.
	Melissa blue	<i>Lycaeides melissa</i> (Edwards)	Unspecified	<i>Lupinus</i> L. spp.
	American copper	<i>Lycaena phlaeas</i> (Linnaeus)	Unspecified	Polygonaceae
	Lustrous copper	<i>Lycaena cupreus</i> (Edwards)	Unspecified	Polygonaceae
	Grey copper	<i>Lycaena dione</i> (Scudder)	Unspecified	Polygonaceae
	Bronze copper	<i>Lycaena hyllus</i> (Cramer)	Unspecified	Polygonaceae
	Ruddy copper	<i>Lycaena rubidus</i> (Behr)	Unspecified	Polygonaceae
Lycaeninae	Blue copper	<i>Lycaena heteronea</i> Boisduval	Unspecified	Polygonaceae
	Bog copper	<i>Lycaena epixanthe</i> (Boisduval and Le Conte)	Unspecified	<i>Vaccinium</i> spp.
	Dorcas copper	<i>Lycaena dorcas</i> Kirby	Unspecified	<i>Potentilla</i> L. spp.
	Maritime copper	<i>Lycaena dospassosi</i> McDunnough	Unspecified	<i>Potentilla</i> spp.
	Purplish copper	<i>Lycaena helleoides</i> (Boisduval)	Unspecified	Polygonaceae
	Lilac-bordered copper	<i>Lycaena nivalis</i> (Boisduval)	Unspecified	Polygonaceae
	Mariposa copper	<i>Lycaena mariposa</i> (Reakirt)	Unspecified	Ericaceae, Polygonaceae

TABLE S2. Ant species in Quebec known to tend Lycaenidae larvae (from: Le guide des papillons du Québec, 1999).

Subfamily	Species
Myrmicinae	<i>Crematogaster lineolata</i> (Say)
Formicinae	<i>Camponotus nearcticus</i> Emery
	<i>Formica oreas</i> Wheeler
	<i>Formica exsectoides</i> Forel
	<i>Formica integra</i> Nylander
	<i>Formica microgyna</i> Wheeler
	<i>Formica rufa</i> Group Linnaeus
	<i>Formica subsericea</i> Say
	<i>Lasius americanus</i> Emery
	<i>Lasius neoniger</i> Emery

NEGATIVE EFFECTS OF EARLY SPRING MOWING ON A BEE COMMUNITY: A CASE STUDY IN THE NIAGARA REGION

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Abstract

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Mowing of bee habitats can change the availability of nesting habitat both above and below ground. Disturbances such as mowing with heavy equipment (“bush-hogging”) that remove all woody vegetation, may affect bees by killing them outright or by altering the availability of nesting and foraging resources. In this case study, we studied the effects of three years (2017 to 2019) of early spring mowing with heavy equipment that removed both herbaceous and woody vegetation from a previously undisturbed, 3 ha meadow (Brock South) in St. Catharines, Ontario, Canada. We predicted that mowing would decrease the abundance of bees that require woody vegetation as their nesting substrate and increase the abundance of below-ground nesters that build nests in exposed soil. We used two approaches to address these predictions. First, using paired, biweekly pan trap collections in 2018 and 2019, we compared bee abundance and diversity from the mowed site (Brock South) to that in an adjacent site of similar size that was unmowed but otherwise very similar (Brock Southwest). Bee abundance and diversity were lower in absolute terms in mowed Brock South than in unmowed Brock Southwest. In mowed Brock South, the carpenter and cavity-nesting bee guilds were lower in relative abundance, while below-ground nesters were higher in relative abundance. Second, we hypothesized that in the absence of mowing, temporal patterns in bee abundance in mowed Brock South should have been like those at undisturbed control sites nearby. Based on pan trap samples collected at three control sites within 1 km of the mowed Brock South site, bee abundance in the absence of disturbance was higher in 2018 and 2019 than in previous years (2008–2017). In contrast, bee abundance in mowed Brock South was not higher in 2018 and 2019 than previous years. Thus, both approaches suggest that even relatively mild disturbances, like mowing once per year, may result in alterations to local bee communities, detectable at small spatial scales of tens to hundreds of metres.

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Introduction

Two kinds of critical resources, floral resources (pollen and nectar) and nesting substrate, directly affect bee abundance. While there is considerable evidence that greater floral abundance leads to greater bee abundance (Roulston and Goodell 2011), how the availability of nesting resources influences bee abundance is not as well understood, at least partly because availability of nesting resource is difficult to measure. Bees exhibit a wide variety of nesting habits. Some construct burrows underground in soil or above ground in substrates like twigs, lumber, or rotting wood. Some construct new cavities, whereas others modify existing holes and crevices.

One way to investigate how the availability of a resource influences the abundance of its consumers is by augmenting or removing it and subsequently observing the effect on local populations. Many landscape management techniques such as plowing, grazing, mowing, and periodic burning, remove or adjust vegetation or soil that bees use as foraging and nesting resources (Buckles and Harmon-Threatt 2019). An immediate effect of mowing is that bees may be killed directly, in their hibernacula, nests, or even on flowers; indirect effects include removal or alteration of nesting and foraging resources (Buri *et al.* 2014; Johansen *et al.* 2019; del Toro and Ribbos 2020). However, in the longer term, mowing can also encourage woody plant growth and floral production of pollen and nectar, increasing both nesting and foraging resources, resulting in increased bee abundance (Lerman *et al.* 2018; Johansen *et al.* 2019). Bee responses to localized disturbances like mowing may vary, depending on the timing and magnitude of the disturbance, but also in ways that reflect the particular traits of the bees themselves (Rutgers-Kelly and Richards 2013; van Klink *et al.* 2019). For instance, when mowing removes above-ground vegetation, above-ground nesters are more likely to be negatively affected than those nesting below-ground, because of the destruction of their nesting substrate (Spiesman *et al.* 2019). Mowing or burning may also adjust the distance between nest sites and foraging sites. Since small-bodied species tend to fly short distances to forage, they may be more severely affected by removal of nesting resources than are large-bodied species that can fly longer distances (Greenleaf *et al.* 2007; Bommarco *et al.* 2010). Similarly, when plowing disturbs the soil, ground-nesting bees with very deep burrows are likely to survive, whereas those in shallow burrows are likely to be killed (Ullmann *et al.* 2016).

The bee fauna of the Niagara Peninsula of southern Ontario, Canada, comprises at least 160 species from five families (Richards *et al.* 2011; Onufko *et al.* 2015). Most Niagara bees are foraging generalists, gathering pollen and nectar from an array of floral species that are abundant in open habitats across the region (Richards *et al.* 2011). In contrast to their generalist foraging habits, Niagara's bee assemblage includes multiple specialized nesting guilds that use different nesting substrates, materials, and locations. The most abundant group in the region are the sweat bees (Halictidae), almost all of which are ground-nesters (Richards *et al.* 2011). Also very abundant in Niagara are the small carpenter bees (*Ceratina* Latreille, Apidae), which construct nests in woody stems and twigs of shrubs such as raspberry (*Rubus* L.), teasel (*Dipsacus fullonum* L.), and sumac (*Rhus typhina* L.) (Rehan and Richards 2010, Vickruck and Richards 2012). Masked bees (*Hylaeus* Fabricius, Colletidae), mason bees (*Osmia* Panzer, Megachilidae) and leafcutter bees (*Megachile* Latreille, Megachilidae) are also very abundant. These do not construct their own burrows,

but seek out holes and crevices, including burrows abandoned by other species, such as *Ceratina*. Another common megachilid is the mason bee *Osmia conjuncta* Cresson, which nests in the empty shells of land snails (Richards *et al.* 2011).

The diversity of nesting habits among Niagara bees suggests that the consequences of landscape alterations should differ among bee species. In this study, we investigated this hypothesis by taking advantage of an acute anthropogenic disturbance conducted by landscape managers at one of our research sites on the campus of Brock University in St. Catharines, Ontario. In the early spring of 2017, an undisturbed meadow site, Brock South, was ‘bush-hogged’, mowed with heavy machinery, removing all vegetation to a height of a few centimetres above ground level. The purpose of the mowing was to remove tall, woody perennials, shrubs and small trees in preparation for a site survey presaging future construction of a stadium in 2020. The mowing was repeated in the springs of 2018 and 2019. After the mowing, flowering plants were left to grow throughout the spring, summer, and fall. We predicted that the main effect of mowing was the removal of nesting substrate rather than nutritional resources, which in turn should lead to a significant decline in the abundance of bees that use woody stems and twigs as nesting substrates, especially the carpenter (e.g. *Ceratina*) and cavity-nesting guilds (e.g. *Hylaeus* and *Megachile*). We also predicted that the use of heavy machinery for mowing would increase the amount of bare soil surface in the field, thus increasing nesting resources for ground-nesting bee species (e.g. *Lasioglossum* Curtis and *Halictus* Latreille).

We used two approaches to examine the effects of mowing on the bee assemblage at the Brock South site. Ideally, to assess the effects of mowing on bee abundance and diversity, we would compare replicated mowed and unmowed sites, before and after mowing. However, this was not possible, given that the mowing of our research site was not discovered until after the fact. We elected to treat this as an opportunity for a case study of the consequences for the local bee community, of a relatively small, localized disturbance that clearly had removed above-ground nesting habitat in the form of woody shrubs and herbaceous stems. We devised two approaches to address the limitations imposed by this unplanned, “natural” experiment. First, in 2018 and 2019, we carried out biweekly pan trap sampling of the bee assemblages of the mowed site, Brock South, and an adjacent, unmowed site, Brock Southwest. These comparisons demonstrated that both bee abundance and diversity were lower in mowed Brock South than in unmowed Brock Southwest. Second, we used comparisons with nearby control sites to assess the likelihood that bee abundance in Brock South was lower after mowing than before, based on the premise that in the absence of mowing, overall bee abundance trends should have been similar across sites.

Materials and methods

Field sites and pan-trapping methods

The focal sites for this study were two adjacent meadows on the southern edge of the Brock University campus in St. Catharines, Ontario ($43^{\circ}6'45''$ N $79^{\circ}14'46''$ W, Figs. 1, 2). At least from 2003 to 2017, neither meadow had been mowed. From 2003–2013, the easternmost of the two sites, Brock South, was monitored as part of a long-term study

(Richards *et al.* 2011, Onufko *et al.* 2018). From 2014 to 2016, Brock South was used for studying the nesting biology of small carpenter bees, *Ceratina*, as the abundance of woody shrubs in this site provided a reliable source of nesting material. On an unknown date in April 2017, the Brock South site was mowed to a grass height of 15 cm and “bush-hogged” to remove woody shrubs and small trees. The site was mowed again on 23 March 2018 (Fig. 2) and on 27 March 2019. After the initial mowing in each year, vegetation was left undisturbed and allowed to regrow.

The Brock Southwest site was directly west of and adjacent to Brock South but was not mowed, and no heavy machinery has been used there in recent years. Brock Southwest contained woody vegetation similar to that in Brock South before the mowing. It was separated from Brock South and protected by a dense hedgerow of trees and shrubs about 14 m wide (Fig. 1). The edges of both sites consisted of dense treeline with forest and a small lake to the south and west and playing fields and parking lots to the north and east. Using the surrounding hedgerows for measurements, the dimensions of Brock South were 233 m × 145 m (3.1 ha) and of Brock Southwest were 271 m × 92 m (2.3 ha) (measured using <https://maps.niagararegion.ca/Navigator/>, accessed December 2020).



FIGURE 1: Satellite images of the unmowed Brock Southwest (BrSW) and mowed Brock South (BrS) sites on the campus of Brock University in St. Catharines in July 2019. The arrow points north. The effects of mowing on BrS are clear in the greater extent of open grassy areas. Using the surrounding hedgerows for measurements, the dimensions of BrS were 233 m × 145 m (3.1 ha) and of BrSW were 271 m × 92 m (2.3 ha). The BrS site had been mowed three times prior to when this photo was taken. Photo was obtained from Google Maps (<https://www.google.ca/maps/@43.1122643,-79.2481538,911m/data=!3m1!1e3!5m1!1e4>).

Pan trapping was used to assess bee population abundance and diversity in both Brock South and Brock Southwest. Pan trapping was carried out simultaneously in both sites using the methods outlined in Richards *et al.* (2013). To summarize, pan traps were 170 g Solo brand PS6-0099 plastic bowls (Solo Cup Company, Lake Forest, IL, USA) filled with water and a small amount of soap to act as a surfactant. Thirty traps were placed on the ground every 3 m in alternating colours (white, yellow, and blue) along a straight transect, 87 metres long. Transects were placed in different areas of each site to get a better representation of the bee assemblage within each field. Traps were placed before 0900 h and collected between 1600 and 1700 h. Trapping was done only on days without rain to ensure bees were actively foraging. Simultaneous pan trapping was carried out approximately biweekly (depending on weather) in both sites from May to September on sunny days without rain. Sampling began in spring with dry, sunny weather, and daily temperatures that reached about 15 °C or more. In late summer, sampling ended when no bees were captured in the traps.

Additional field sites

Since neither Brock South nor Brock Southwest was surveyed prior to the mowing events, we could not examine bee abundance before and after mowing in these two sites. Instead, we compared the Brock South and Brock Southwest sites to three undisturbed sites (Esc, Pon, and Summit) at the Glenridge Quarry Naturalization Site (GQNS), about 900 m away (straight-line distance), based on the premise that environmental conditions at GQNS



FIGURE 2: Ground-level photos of Brock South (BrS) and Brock Southwest (BrSW) in April 2018. All shrubs and woody vegetation had been removed from BrS and the grass was cut short.

should be very similar to those south of the Brock campus. All three GQNS sites were grassy meadows, and Esc and Pon were described previously (Rutgers-Kelly and Richards 2013, Onuferko *et al.* 2018). The Summit site ($43^{\circ}7'19''$ N $79^{\circ}14'10''$ W) was first used in 2015 and is located at the top of the central hill in the middle of the GQNS. Pan trapping methods at the GQNS were essentially identical to those used at Brock South and Brock Southwest, with approximately biweekly surveys, usually from May to September (Table 1; Rutgers-Kelly and Richards 2013, Onuferko *et al.* 2018).

Bee identification

Bees trapped in 2018 and 2019 were stored in 70% ethanol, then pinned and labelled with the site location, date, and trapping method. Bees were identified to species using the following taxonomic keys: Mitchell 1960, Packer *et al.* 2007, Rehan and Richards 2008, Gibbs 2010, Gibbs *et al.* 2011, Gibbs *et al.* 2013, Sheffield *et al.* 2011a, Sheffield *et al.* 2011b, and online, multi-chotomous keys at discoverlife.org (Ascher and Pickering 2020). *Andrena* Fabricius were identified only to genus due to difficulties with identification. *Hylaeus affinis* (Smith) and *H. modestus* Say, *Ceratina mikmaqi* Rehan and Sheffield and *C. dupla* Say, and bidentate species of *Nomada* Scopoli were combined into three morphospecies, respectively. Nine specimens were damaged and identified only to genus; these were not included in counts of morphospecies richness. Initial specimen sorting to morphospecies was carried out by the first author (TA) and all specimens were identified to species by the second author (NR), with confirmations for a subset of difficult groups such as *Lasioglossum* subgenus *Dialictus* Robertson by Dr. Thomas Onuferko. All specimens are currently stored in the collection of the Brock Bee Lab at Brock University in St. Catharines. Nesting guild information was obtained from Cane *et al.* (2007) and Onuferko *et al.* (2015).

Data analysis

Week numbers were assigned using the WEEKNUM function in Excel, setting the last week of April to week 1. Statistical analyses were carried out in R, version 3.6.3, running under the R-Studio shell, version 1.0.143 (RStudio Team 2020).

In week 3 of 2019, we could not collect bees from the Brock South site due to construction activity, so we excluded week 3 from all analyses. All remaining collections in Brock South (BrS) and Brock Southwest (BrSW) in 2018 and 2019 were done simultaneously, allowing direct comparisons of bee abundance, measured as the number of bees caught per biweekly collection (a collection is the entire set of bees caught in 30 traps over the course of one day). We used a general linear model of the form $\text{Abundance} = \text{Site} + \text{Week} * \text{Year}$ (where * denotes an interaction term) to compare Brock South and Brock Southwest, because bee abundance and community composition are known to change significantly from week to week due to differences in phenology among local families and guilds (Onuferko *et al.* 2018). Because sample size is itself a cause of apparent differences in the species richness of two samples (Richardson and Richards 2008), we used rarefaction curves as implemented in the R *vegan* package, *rarecast* function, to compare species richness in 2018, 2019, and both years pooled. Rarefaction methods generate different-sized, random subsamples of a dataset, and count the number of species or morphospecies in each sample. Guild composition of the bee assemblages in Brock South and Brock Southwest was compared in terms of the relative proportions of bees in each guild category, using χ^2 tests of homogeneity.

TABLE 1. Numbers of pan trapping collections that captured at least one specimen at all research sites used in this study, 2008–2019. There was no trapping at these sites in 2014 or 2016. BrS was mowed in 2017, 2018, and 2019 while all other sites were undisturbed (indicated by *M*). Esc, Pon, and Summit are three long-term monitoring sites undisturbed since 2003. All sites were pan trapped with identical methods and schedules. Within a year, the numbers of collections varied slightly due to inclement weather or because construction schedules prevented trapping.

Year	Sampling dates	Site			
		BrS (mowed)	BrSW (unmowed)	Esc (control)	Pon (control)
2008	22 Apr.–24 Sep.	22			18
2009	5 May–29 Sep.	18		18	16
2010	21 Apr.–30 Aug.			8	
2011	25 May–8 Oct.	10		10	10
2012	22 Mar.–20 Sep.	12		10	11
2013	1 May–2 Oct.	12		13	11
2015	18 May–4 Sep.			9	9
2017	10 May–26 Sep.	(<i>M</i>)		10	10
2018	8 May–28 Sep.	11 (<i>M</i>)	11	10	
2019	6 May–13 Sep.	8 (<i>M</i>)	9		8

To assess temporal change in bee abundance at the control sites near Brock South, we analysed pan trap abundance at the three control sites during two time phases, “Before” (2008 to 2017) and “After” (2018 to 2019). Demographic patterns may vary among bee families and since collecting effort varied among sites and years (Table 1), we calculated the average annual number of bees of each family per collection (total number of bees collected at a site each year / number of collections in that same site each year). This generated a matrix of estimated bee abundance for each family (5 columns) for each of 18 site-by-year combinations (18 rows). This matrix was then analysed using permutational multivariate analysis of variance (permanova), as implemented in the R *vegan* package (version 2.5-6), employing a model of the form [Abundance matrix] = Phase, with Site as a random factor.

Results

Effects of mowing, 2018 and 2019

Table 2 presents the complete list of bee species collected via pan traps in the mowed Brock South and unmowed Brock Southwest sites in 2018 and 2019. Most species collected had been previously identified in the region (Onufreko *et al.* 2015), but the megachilid, *Osmia bucephala*, represents a new record for Niagara.

TABLE 2. Complete list of bee species and morphospecies collected in the mowed BrS and unmowed BrSW sites in 2018 and 2019 (excluding week 3 of 2019). The three *Lasioglossum* subgenera are *Lasioglossum* (*La.*), *Leuchalictus* (*Le.*), and *Dialictus* (*D.*). Species marked with an asterisk (*) were unidentifiable due to damage. SR = morphospecies richness. Nesting guilds are as follows: Ground = digs underground burrows, Cavity = nests in pre-existing cavities, Carpenter = excavates nests from pith within stems or in wood (or in oak-apple galls, as is the case with *O. simillima*), Parasite = lays eggs in nests of other bee species, Rotting wood = nests under the bark or inside of rotting stumps, Snail shell = nests in the empty shells of land snails.

Family and species	BrS (mowed)		BrSW (unmowed)		Nesting guild
	2018	2019	2018	2019	
Andrenidae					
<i>Andrena cressonii</i> Robertson	1	0	1	0	Ground
<i>Andrena</i> sp.	2	0	6	2	Ground
Family abundance (SR)	3 (2)	0 (0)	7 (2)	2 (1)	
Apidae					
<i>Apis mellifera</i> Linnaeus	0	5	0	12	Cavity
<i>Bombus borealis</i> Kirby	0	0	1	0	Ground
<i>Bombus griseocollis</i> (DeGeer)	1	0	0	0	Ground
<i>Bombus impatiens</i> Cresson	1	0	2	0	Ground
<i>Bombus rufocinctus</i> Cresson	3	1	4	0	Ground
<i>Ceratina calcarata</i> Robertson	17	9	46	42	Carpenter
<i>Ceratina dupla/mikmaqi</i>	27	30	54	68	Carpenter
<i>Melissodes druriella</i> (Kirby)	0	0	1	2	Ground
<i>Nomada denticulata</i> Robertson	0	0	0	1	Parasite
<i>Xylocopa virginica</i> (Linnaeus)	0	0	1	0	Carpenter
Family abundance (SR)	50 (5)	45 (4)	111 (7)	125 (5)	
Colletidae					
<i>Hylaeus affinis/modestus</i>	16	14	20	20	Cavity
<i>Hylaeus annulatus</i> (Linnaeus)	2	0	1	7	Cavity
<i>Hylaeus hyalinatus</i> Smith	0	0	1	0	Cavity
<i>Hylaeus illinoiensis</i> (Robertson)	1	0	0	6	Cavity
<i>Hylaeus</i> sp.*	0	0	0	1	Cavity
Family abundance (SR)	19 (3)	14 (1)	22 (3)	34 (4)	
Halictidae					
<i>Agapostemon virescens</i> (Fabricius)	2	0	5	1	Ground
<i>Augochlora pura</i> (Say)	0	0	1	1	Rotting wood
<i>Augochlorella aurata</i> (Smith)	58	60	76	36	Ground
<i>Halictus confusus</i> Smith	8	11	19	32	Ground
<i>Halictus ligatus</i> Say	50	50	111	79	Ground
<i>Halictus rubicundus</i> (Christ)	2	1	4	0	Ground
<i>Lasioglossum</i> (<i>La.</i>) <i>coriaceum</i> (Smith)	0	0	0	1	Ground
<i>Lasioglossum</i> (<i>D.</i>) <i>cressonii</i> (Robertson)	0	0	0	1	Ground
<i>Lasioglossum</i> (<i>Le.</i>) <i>leucozonium</i> (Schrank)	4	3	2	4	Ground
<i>Lasioglossum</i> (<i>D.</i>) <i>admirandum</i> (Sandhouse)	7	4	0	0	Ground

TABLE 2 continued...

<i>Lasioglossum (D.) atwoodi</i> Gibbs	4	3	5	3	Ground
<i>Lasioglossum (D.) hitchensi</i> Gibbs	56	9	11	14	Ground
<i>Lasioglossum (D.) lineatulum</i> Crawford	0	0	0	1	Ground
<i>Lasioglossum (D.) nymphaearum</i> (Robertson)	2	9	1	2	Ground
<i>Lasioglossum (D.) oblongum</i> (Lovell)	0	0	0	1	Ground
<i>Lasioglossum (D.) versatum</i> (Robertson)	9	13	11	25	Ground
<i>Lasioglossum (Le.) zonulum</i> (Smith)	1	0	0	0	Ground
<i>Lasioglossum (D.)</i> sp.*	4	0	4	0	Ground
Family abundance (SR)	207 (12)	163 (10)	250 (11)	201 (14)	
Megachilidae					
<i>Anthidium manicatum</i> (Linnaeus)	0	1	3	2	Cavity
<i>Anthidium oblongatum</i> (Illiger)	0	0	12	3	Cavity
<i>Hoplitis pilosifrons</i> (Cresson)	1	2	1	3	Cavity
<i>Hoplitis producta</i> (Cresson)	0	3	1	5	Cavity
<i>Hoplitis spoliata</i> (Provancher)	1	1	1	1	Cavity
<i>Megachile brevis</i> Say	3	5	5	0	Cavity
<i>Megachile campanulae</i> Robertson	0	0	0	2	Cavity
<i>Megachile centuncularis</i> (Linnaeus)	0	1	0	0	Cavity
<i>Megachile ericetorum</i> Lepeletier	1	0	1	0	Cavity
<i>Megachile gemula</i> Cresson	0	0	1	0	Ground
<i>Megachile latimanus</i> Say	1	0	0	0	Ground
<i>Megachile relativa</i> Cresson	1	0	4	2	Cavity
<i>Megachile rotundata</i> (Fabricius)	0	1	1	0	Cavity
<i>Osmia atriventris</i> Cresson	1	2	6	2	Cavity
<i>Osmia bucephala</i> Cresson	4	0	11	0	Cavity
<i>Osmia conjuncta</i> Cresson	68	5	49	6	Snail Shell
<i>Osmia pumila</i> Cresson	3	1	15	3	Cavity
<i>Osmia simillima</i> Smith	1	0	0	0	Carpenter
<i>Stelis lateralis</i> Cresson	0	0	0	1	Parasite
Family abundance (SR)	85 (11)	22 (10)	111 (14)	30 (11)	
Total abundance (SR)	364 (33)	244 (25)	501 (37)	390 (34)	

Overall, biweekly bee abundance was lower in mowed Brock South than in unmowed Brock Southwest, particularly in the latter half of the summer (Fig. 3), and the difference between sites was highly significant and consistent between years (Table 3). Not only was bee abundance lower in mowed Brock South, but bee diversity, measured as morphospecies richness, was also lower. In 2018, mowed Brock South yielded 33 species, while unmowed Brock Southwest had 37. In 2019, mowed Brock South had 25 species, while unmowed Brock Southwest had 34. Thus, the divergence in species richness between mowed and unmowed sites seems to have increased over time. This is further illustrated in the rarefaction curves (Fig. 4), which show that the lower morphospecies richness in mowed Brock South was not simply due to lower bee abundance there. These comparisons suggest that spring mowing led to a decrease in both abundance and diversity of the bee assemblage in Brock South.

Comparisons between mowed Brock South and unmowed Brock Southwest also

revealed differences in community composition (Table 4). Most noticeable was the lower relative abundance in Brock South of carpenters and cavity-nesters, contrasting with the higher abundance of ground-nesters, changes that were predicted due to changes in nesting substrate availability for these two guilds. Also of note, snail shell-nesters, represented by the very abundant species, *Osmia conjuncta*, were proportionately twice as abundant in mowed Brock South as in unmowed Brock Southwest in 2018 (the omission of week 3 from 2019, prevented comparison in 2019, since most *O. conjuncta* are found in early May).

Abundance trends before and after 2018

Average annual abundance of each of the five bee families before (2008 to 2017) and after mowing (2018 to 2019) is compared in Fig. 5. In the undisturbed control sites, bee abundance was significantly higher in 2018 and 2019 than in the preceding years (permanova, $R^2 = 0.247$, $F_{1,16} = 5.253$, $P = 0.006$). This suggests that in the absence of disturbance, bee abundance in mowed Brock South should also have been higher in 2018 and 2019 than in previous years, but the abundance of bees in each of the five families was not significantly different in 2018 and 2019 than in previous sampling years (Permanova, $R^2 = 0.061$, $F_{1,5} = 0.327$, $P = 0.86$). That bee abundance in the mowed Brock South site failed to increase in 2018 and 2019, is consistent with the hypothesis that bee abundance was lower after mowing than it would have been without mowing.

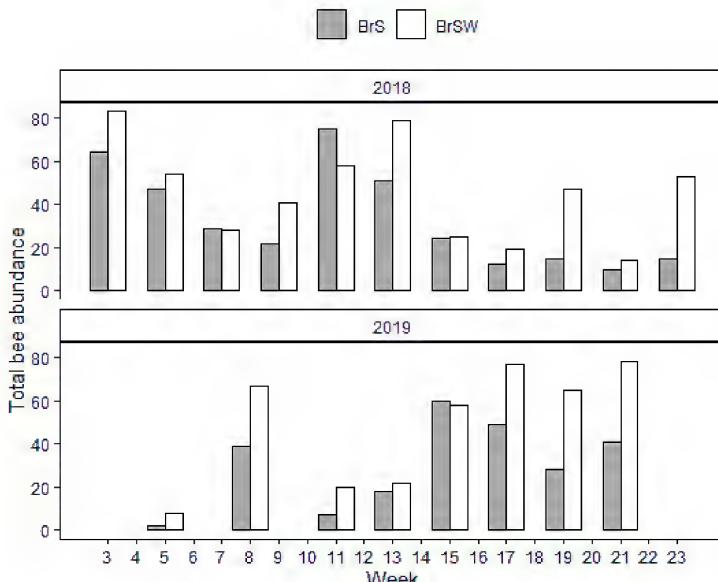


FIGURE 3: Comparison of bee abundance in paired pan trap collections at BrS and BrSW in 2018 and 2019. Note that in week 3 of 2019, only the BrSW site was sampled, so these data were omitted from the comparison.

TABLE 3. Results for general linear model analysing variation in bee abundance (total number of bees per collection) in BrS and BrSW in 2018 and 2019. The Site term indicates significantly higher bee abundance in BrSW than in BrS. Differences among weeks are due to differences in bee phenology over time. There was no significant effect of Year (2018 vs. 2019), although there was a significant interaction between Week and Year (see Fig. 3).

Source	Degrees of freedom	F value	P (>F)
Site	1	21.86	0.0002
Week	11	4.09	0.0042
Year	1	1.57	0.226
Week * Year interaction	6	24.53	<0.0001
Residuals	18		

This case study suggests that even limited disturbance, mowing a single field once per year in early spring, was enough to influence the abundance and composition of the bee assemblage foraging and nesting in that field. Overall, the mowed site (Brock South) had lower bee abundance and diversity than the unmowed site, suggesting that mowing exerted a negative impact through loss of nesting substrate or food resources (or both). In fact, the observed differences between the mowed and unmowed sites may underestimate the differences between them. The two sites were adjacent fields about three hectares in area, which suggests that the bee assemblages in these two fields were spatially differentiated on a scale of only ten to hundreds of metres. This in turn implies that the bees foraging and nesting in these fields might be responding to nesting and nutritional resources at quite a small spatial scale. This seems especially likely for small-bodied bees like *Ceratina* or *Lasioglossum (Dialictus)*, which likely have short, breeding dispersal distances from their natal nests and short commutes from their nests to foraging sites (Greenleaf *et al.* 2007). Moreover, the unmowed and mowed sites were separated by a thick hedgerow, which remained undisturbed by mowing and could have provided considerable bee nesting habitat, especially for carpenters and cavity-nesters. These bees could have foraged in either the mowed or unmowed fields, which would have obscured differences in bee abundance between fields. One reason for moving the pan trap transects around the sites was to avoid systematic spatial errors that might be associated with over-collecting bees with very short foraging distances (Greenleaf *et al.* 2007). Taken together, these considerations suggest that the significant differences detected here likely underestimate the differences in bee assemblages between the two sites.

Differences between the mowed and unmowed sites in the proportional representation of the different nesting guilds suggest trait-specific responses of bees to disturbance (Klink *et al.* 2019). Mowing certainly reduced the availability of nesting substrate for the carpenter and cavity-nester guilds and likely also altered access to nesting substrate for ground nesters and possibly snail shell nesters. At the same time, the mowed site had proportionately more ground-nesters than the unmowed site. If mowing had directly increased the availability of open soil surface for ground-nesters, attracting more ground-

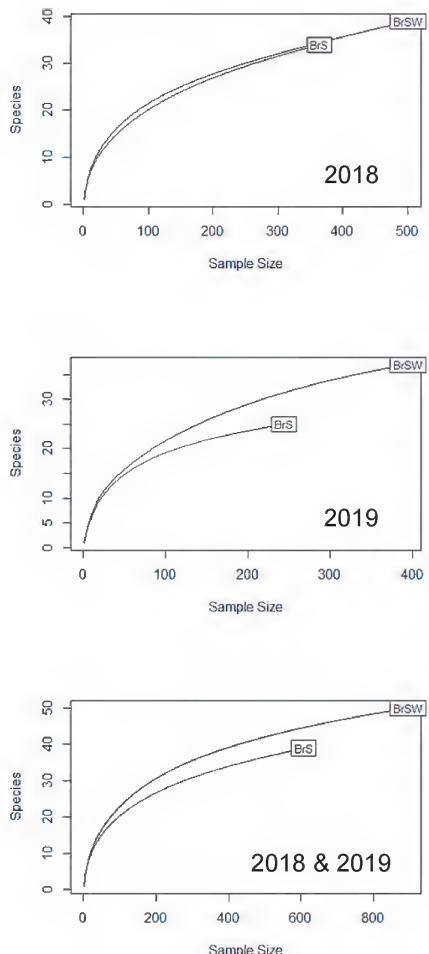


FIGURE 4: Rarefaction curves comparing the number of species expected in subsamples of pan trap collections from mowed BrS versus unmown BrSW, in 2018, 2019, and in both years pooled.

nesters like *Lasioglossum* into the Brock South area, then we would have expected an increase in their absolute numbers, not just an increase in relative abundance. It seems most likely that removal of above-ground nesting substrate was the main reason for the decline in the carpenter and cavity-nesting guilds, creating an apparent increase in the proportion of ground nesters. One difference not predicted between mowed and unmown sites was the higher proportion of snail-shell nesters (*Osmia conjuncta*) in the mowed site in 2018. It is possible that mowing reduced the vegetational cover for snails, exposing them to higher mortality risk due to high temperature exposure and desiccation risk, and thus increasing the availability of shells for *O. conjuncta* to nest in.

The higher bee abundance in the control sites in 2018 and 2019 suggests that in the absence of disturbance, local bee populations were generally on the increase. However, these increases at nearby sites were not matched by an increase in bee abundance at the mowed site, Brock South. This provides additional evidence that after mowing, bee abundance was lower in Brock South than it would have been if the site had not been mowed. Exactly why abundance declined is unclear, but either mortality or migration could have played a part. Many carpenter and cavity-nesting bees that were in nests or hibernacula during the early spring mowing of 2017 likely died as a direct result of being crushed during mowing operations. Survivors could have moved to nearby fields and meadows with appropriate nesting substrate, including Brock Southwest, since the maximum distance from Brock South to neighbouring areas was just a

TABLE 4. Nesting guild abundance in BrS (mowed) and BrSW (unmowed) sites in 2018 and 2019. For each guild, numbers represent the number of specimens collected, and percentages represent proportional representation at each site. The data for the parasite and rotting wood guilds were excluded from the chi-square tests because of small sample sizes.

Nesting guild	2018		2019	
	BrS	BrSW	BrS	BrSW
Carpenter	45 (12.4%)	101 (20.3%)	39 (16.0%)	110 (28.1%)
Cavity	34 (9.4%)	83 (16.7%)	36 (14.8%)	69 (17.6%)
Ground	216 (59.5%)	265 (53.2%)	164 (67.2%)	207 (52.8%)
Snail shells	68 (18.7%)	49 (9.8%)	5 (2.0%)	6 (1.5%)
χ^2 test, df = 3	29.639, $P < 0.0001$		15.688, $P = 0.0013$	

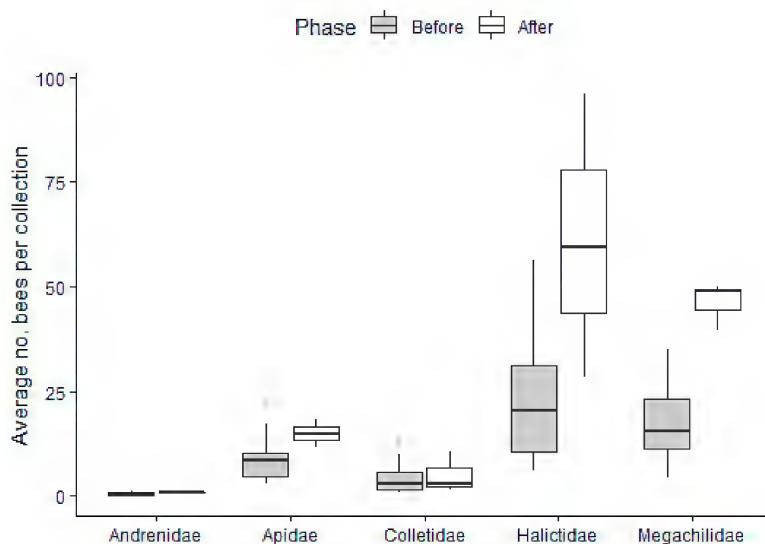


FIGURE 5: Changes in the average abundance of each bee family at three control sites during the two time phases before (2008 to 2017, grey) and after (2018 and 2019, white) mowing at the BrS site. See Table 1 for annual pan trapping schedules at the control sites.

little more than 233 m. Moreover, after mowing, the Brock South site would probably not have attracted as many carpenters or cavity-nesters, given the lack of nesting substrate. The lower absolute abundance of this guild in mowed Brock South compared to unmowed Brock Southwest, was therefore most likely due to a combination of mortality due to mowing and lack of nesting resources to attract immigrants. If mowing also decreased floral abundance in Brock South, then this is another reason that fewer foragers would have been attracted to the site.

The lower absolute abundance of ground nesters in Brock South is harder to explain than the lower abundance of carpenters and cavity-nesters. It is possible that mowing did not improve access to bare soil patches, or that the movement of heavy equipment may have compacted the soil, making it less suitable for sweat bees and other ground nesters (Buckles and Harmon-Threatt 2019). If as suggested above, mowing actually decreased floral abundance, then there would have been fewer foragers in the mowed site and thus fewer bees caught in pan traps.

Conclusion

Whether mowing killed bees directly or induced them to move elsewhere, this case study demonstrates that bee populations may respond negatively to relatively low levels of disturbance, and that this response is detectable at a small spatial scale. Conversely, previous studies of bee abundance and diversity in Niagara have demonstrated positive responses of bee populations to habitat restoration; after extremely severe disturbances that eradicated local bee populations for decades, habitat restoration at the Glenridge Quarry Naturalization Site resulted in rapid and sustained recovery of bee abundance and diversity within only three to five years (Rutgers-Kelly and Richards 2013; Onufko *et al.* 2018). It appears that bee populations respond rapidly to changes in the availability of critical resources (Tscharntke *et al.* 1998; Winfree *et al.* 2009; Spiesman *et al.* 2019), suggesting considerable variability in bee abundance and diversity at spatial and temporal scales both small and large.

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WILD BEES (HYMENOPTERA: APOIDEA) FROM REMOTE SURVEYS IN NORTHERN ONTARIO AND AKIMISKI ISLAND, NUNAVUT INCLUDING FOUR NEW REGIONAL RECORDS

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Abstract

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Bees (Hymenoptera: Anthophila) are important pollinators in northern Canada but observations of species occurrences in such remote regions are largely lacking. We report on bee species surveyed across 22 sites in northern Ontario and neighbouring Akimiski Island in Nunavut, using coloured pan traps, Nzí traps, Malaise traps, and hand netting over five years in Ontario and nine years in Nunavut. From morphological identification and DNA barcoding, we identified 30 species from 93 specimens. We document four new occurrence records for Nunavut, *Halictus virgatellus* Cockerell (Halictidae), *Megachile circumcincta* (Kirby) (Megachilidae), *Megachile melanophaea* Smith (Megachilidae), and *Osmia paradisica* Sandhouse (Megachilidae). Also, we report on a bilaterally gynandromorphic *M. melanophaea* from Akimiski Island and a new DNA barcode for the rare *Osmia nearctica* Rightmyer, Griswold and Arduser (Megachilidae). These northern collections represent one of the most comprehensive surveys of wild bees in the southern Hudson Bay region, filling an important biogeographic gap for Canada.

Introduction

Bees (Hymenoptera: Apoidea) are important pollinators in boreal ecosystems (Kevan 1972; Kevan *et al.* 1993). Understanding the contemporary distribution of bee species

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is necessary to predict how climate change and resource extraction might alter the diversity and pollination services of bee communities, especially in northern regions (Vernier *et al.* 2014). Studies from the northern Palearctic Region suggest that many bee species might be extending their ranges northward (Franzén and Ockinger 2012; Kratochwil 2016). To detect similar range shifts in the Nearctic Region, we first need baseline distribution data, which are lacking for many parts of northern Canada, including northern Ontario.

Historic surveys in this region occurred along the coast of Hudson Bay and James Bay, at Fort Severn, Fort Albany, and Moosonee in Ontario, as well as the west coast of Quebec and along the Great Whale River (Danks 2004). Those surveys were conducted by staff of the Canadian National Collection of Insects as part of the Northern Insect Survey from 1947–1958 (see maps in Freeman and Twinn 1954; Huckett 1965). Previous pollinator surveys of northern Ontario focused on fly (Diptera) pollinators (Kevan 1972; Vezsenyi *et al.* 2021). In comparison, few surveys in northern Canada report on wild bee communities outside of western Canada (Sakagami and Toda 1986; but see Provancher 1888; Gibson *et al.* 2018); however, all of northern Canada is data deficient due to how large and remote this area is, making it challenging to comprehensively survey.

Here, we report on wild bee species collected during a large-scale survey that targeted biting flies specifically due to their impact on wildlife, from remote parts of northern Ontario and nearby Akimiski Island, Nunavut. Our report excludes bumble bees, *Bombus* Latreille spp. (Apidae), which were described in Gibson *et al.* (2018).

Surveys in northern Ontario were undertaken with assistance from the Ontario Ministry of Natural Resources and Forestry (MNRF) Far North Biodiversity project (FNPB) (Fig. 1), a six-year project to survey a range of terrestrial plant and animal species. The FNPB represented the first known insect surveys for many of these sites, most of which were accessible only by helicopter. As part of the FNPB, we surveyed north of Ontario's Far North line (Far North Act, S.O. 2010, c.18 s.2; Fig. 1), a region that covers 451,808 km² and is divided into two ecozones. The Hudson Bay Lowlands ecozone is an area dominated by peatlands, bogs, fens, and small lakes, with stunted tamarack (*Larix* Mill. spp., Pinaceae) and black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenburg, Pinaceae) as the primary vegetation in drier areas. Peat moss (*Sphagnum* L. spp., Sphagnaceae) mats and sedges (*Carex* L. spp., Cyperaceae) characterize the wetter areas (Government of Ontario 2020). The Boreal Shield ecozone is dominated by black spruce, jack pine (*Pinus banksiana* Lamb., Pinaceae), balsam fir (*Abies balsamea* (L.) Mill., Pinaceae), birch (*Betula* L. spp., Betulaceae), and balsam poplar (*Populus balsamifera* L., Salicaceae) (Crins *et al.* 2009). These surveys were documented in detail in the Far North Science Advisory Panel (2010) and Ringrose *et al.* (2013).

Surveys on Akimiski Island in James Bay were completed in cooperation with the MNRF Wildlife Division. Akimiski Island (Fig. 2A) is a 3,800 km² island that re-emerged 5,000 years ago after the end of the last glacial period (Martini 1981). The island comprises a variety of habitats, with a maritime climate where temperature extremes are moderated by James Bay. Part of the interior is covered in coniferous forest, and the rest is peatland with patches of rocky tundra habitat comparable to the Hudson Bay Lowlands (Klinger and Short 1996; Vezsenyi *et al.* 2019).

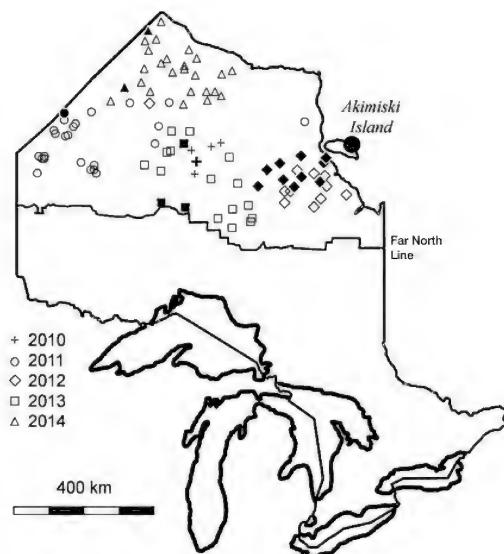


FIGURE 1. Map depicting field camps where bees were collected (closed/bolded symbols) or not collected (open/not bolded) during the Far North Biodiversity project in northern Ontario, and sampling on Akimiski Island (large opaque circle, representing samples from 2008–2017). All surveys were conducted above the Far North line.



FIGURE 2. (A) The research station on Akimiski Island, Nunavut. Traps were deployed in the helicopter landing strip to the right of the white buildings when the strip was not in use. Additional photos are of (B) an Nzi trap and (C) a Malaise trap deployed on Akimiski Island (same as used in northern Ontario), (D) representative vegetation in the Akimiski Island interior, and (E) an example of the risks when sampling in northern environments. Photographs 2B–D by David Beresford and photographs 2A, E by Kathryn Vezsenyi.

Materials and methods

The FNBP terrestrial survey in northern Ontario occurred between June and July from 2010–2014 at >400 distinct sites distributed across 91 temporary field camps (Fig. 1). Sites were predetermined along intersecting transect lines that extended outward from field camps. Each site was visited once and surveyed daily for seven days within a particular calendar year (Fig. 1). Insects, including bees, were collected using four methods: pan traps used to catch flower-visiting insects [shallow, 5-oz dishes painted one of three colours of Rust-Oleum Tremclad spray paint: white (colour code: RAL 9010), yellow (RAL 1026), and light blue (RAL 5012)], Nzi traps (a biting fly trap, Fig. 2B; Mihok 2002), Malaise traps (Fig. 2C), and by hand netting once per day. Nine pan traps (three of each colour) were deployed near the field camp in an ‘X’ shape, spaced a minimum 2.25 m apart where there was sufficient bare ground (Gan *et al.* 2009). Pans were filled with a propylene glycol mixture consisting of 50% water and 50% non-toxic antifreeze (Starbrite RV Anti-freeze). At each field camp, if there was a dry, open-canopied area (e.g., not in a densely wooded landscape or submerged in water), an Nzi trap and Malaise trap were placed nearby. Both traps were outfitted with collecting heads filled with 80% denatured ethanol (Ringrose *et al.* 2014). All traps were emptied each day, and captured insects were stored in 500 ml sample jars in 80% ethanol.

Specimens from Akimiski Island were collected by DVB from mid-July to early August of 2008–2017. Sampling took place at the research station (53°04'02"N, 80°58'14"W; Fig. 2D) using pan, Nzi, and Malaise traps deployed from dawn to dusk each day (minimum 2.25 m apart within a fenced camp area to protect researchers from polar bears (*Ursus maritimus* Phipps, Ursidae) (Fig. 2E). Pan traps were filled with propylene glycol (as in northern Ontario) but because these were remote sites with limited access, soapy water was used in some deployments. Sweep netting of vegetation for general insect surveying was conducted *ad hoc* in the surrounding vicinity outside the fence, and farther away when opportunities arose. Captured specimens were stored in 80% ethanol and grouped by trap type and catch date.

Identification and curation

Collections were processed at Trent University and identified to genus by KMV using Packer *et al.* (2007) and Ascher and Pickering (2020), then to species by KLJH using identification keys [Mitchell (1962) and Ascher and Pickering (2020) for *Anthophora* Latreille, *Coelioxys* Latreille, and *Hoplitis* Klug; Sheffield *et al.* (2011) for *Megachile* Latreille; Rightmyer *et al.* (2010) and additional consultation with M. Rightmyer for *Osmia* Panzer; Laberge and Ribble (1975), Laberge (1980), and Laberge (1986) for *Andrena* Fabricius; Mitchell (1960) and Roberts (1973) for *Halictus* Latreille; Gibbs (2010) and Gibbs *et al.* (2013) for *Lasioglossum* Curtis; and Romankova (2003), Stephen (1954), and Mitchell (1960) for *Colletes* Latreille], and referencing specimens in the Packer Collection at York University, the Royal Ontario Museum, and the Biodiversity of Urban Green Spaces ('BUGS') Lab at the University of Toronto Scarborough. We additionally attempted to obtain sequences of the cytochrome c oxidase subunit I (CO1) gene (DNA barcodes) for all specimens following standard protocols (Canadian Centre for DNA Barcoding, Ivanova *et al.* 2006). We compared the resulting sequences to those uploaded to the Barcode of Life

Data (BOLD) System and cross-referenced sequences in GenBank (Benson *et al.* 2018) using the Basic Local Alignment Search Tool (BLAST, Zhang *et al.* 2000). We used $\geq 98\%$ genetic matches to assign species identities (Packer *et al.* 2009). The goal of obtaining CO1 barcodes was to confirm taxonomy-based determinations in cases where pre-existing barcodes were available and to contribute novel barcodes for species and geographical populations not yet represented in barcode libraries. The full list of bee species, their abundances, and GenBank accession numbers are available in Table S1. Descriptions of life history traits (pollen specialization, sociality, and nesting preference) for each species are included in Table S2. We summarize the known provinces and territories of occurrence for each species based on published collections and peer-reviewed literature in Table S3. Georeferenced species occurrence records were uploaded to Canadensys for databasing on the Global Biodiversity Information Facility (GBIF) (Vizza *et al.* 2021). All bee specimens are curated in the BUGS Lab at University of Toronto Scarborough.

Results

We collected 93 bee specimens belonging to 30 species: 23 species from northern Ontario (FNBP) and 16 species from Akimiski Island, with 9 species occurring in both (Table 1). In Ontario, 56.5% (13/23) of species were singletons, compared with 43.8% (7/16) of species on Akimiski Island (Table 1). In Ontario, bees were collected at 22 sites across 19 field camps (Table S1). The most common bee species was *Osmia nigriventris* (Zetterstedt) (Megachilidae) in Ontario ($N = 5$) and *Halictus virgatellus* Cockerell (Halictidae) on Akimiski Island ($N = 14$). No patterns in life history traits were evident; species were mostly polyleptic and solitary, with 57% ($N = 17$) nesting in soil, 33% ($N = 10$) in pre-existing cavities, one cleptoparasite, and two species with unknown nesting preferences (*Osmia nearctica* and *O. paradisica*) (see Table S2).

Among the specimens collected, we found four new species records for Nunavut (Fig. 3): *H. virgatellus*, *Megachile circumcincta* (Kirby) (Megachilidae), *Megachile melanophaea* Smith (Megachilidae) (including one bilateral gynandromorph; Fig. 4) and *Osmia paradisica* Sandhouse (Megachilidae). Our record of *M. circumcincta* further represents the easternmost known occurrence of this species within North America documented to date, extending its range eastward by approximately 1,050 km.

We extracted sufficient DNA from 68 of 93 bee specimens for high-quality sequences whose congruence with previously deposited sequences corroborated our morphological identifications for 24 species (Table 1). For 23 specimens belonging to 14 species, we were unable to extract DNA of sufficient quality to be assigned a Barcode Index Number (BIN) (Table S1). For five of these species [*Colletes consors* Cresson (Colletidae), *Halictus confusus* Smith (Halictidae), *Lasioglossum novascotiae* (Mitchell) (Halictidae), *Osmia bucephala* Cresson (Megachilidae), and *Osmia laticeps* Thomson (Megachilidae)] we were unable to corroborate morphological identifications using CO1 barcodes. Additionally, we obtained high-quality CO1 sequences for two specimens of *O. nearctica* (Megachilidae), thereby contributing the first barcodes for this species for BOLD and GenBank. Because no prior sequence for this species was available, BLAST incorrectly identified the sequences as *Osmia nigrifrons* Cresson (Megachilidae) at a 98.7%

TABLE 1. Species listed by family, subgenus, and number collected in northern Ontario and Akimiski Island, Nunavut (see Table S2 for life history traits for each species). First records for the territory of Nunavut are identified with an asterisk* whereas a caret^ denotes records new to both Nunavut and Ontario.

Family	Subgenus	Species	Ontario	Akimiski	Total
Apidae	<i>Clisodon</i>	<i>Anthophora terminalis</i> Cresson		1	1
Megachilidae	<i>Coelioxys</i>	<i>Coelioxys sodalis</i> Cresson	2	1	3
	<i>Alcidamea</i>	<i>Hoplitis albifrons</i> (Kirby)	2		2
	<i>Megachile</i>	<i>Megachile lapponica</i> Thomson	3	2	5
		<i>Megachile relativa</i> Cresson		1	1
	<i>Xanthosarus</i>	<i>Megachile circumcincta</i> (Kirby)*		1	1
		<i>Megachile frigida</i> Smith	2	2	4
		<i>Megachile gemula</i> Cresson	1	2	3
		<i>Megachile melanophaea</i> Smith*		3	3
		<i>Megachile perihirta</i> Cockerell		3	3
	<i>Melanosmia</i>	<i>Osmia bucephala</i> Cresson	1	1	2
		<i>Osmia laticeps</i> Thomson	1		1
		<i>Osmia nearctica</i> Rightmyer, Griswold & Arduser	3		3
		<i>Osmia nigriventris</i> (Zetterstedt)	5	2	7
		<i>Osmia paradisica</i> Sandhouse*		8	8
		<i>Osmia tarsata</i> Provancher	1		1
		<i>Osmia tersula</i> Cockerell	1		1
Andrenidae	<i>Andrena</i>	<i>Andrena frigida</i> Smith	1		1
	<i>Euandrena</i>	<i>Andrena algida</i> Smith	1		1
	<i>Leucandrena</i>	<i>Andrena barbilabris</i> (Kirby)	1		1
Halictidae	<i>Protohalictus</i>	<i>Halictus rubicundus</i> (Christ)	2		2
	<i>Seladonia</i>	<i>Halictus confusus</i> Smith	1		1
		<i>Halictus virgatellus</i> Cockerell^	2	14	16
	<i>Sphecodogastera</i>	<i>Lasioglossum boreale</i> Svensson, Ebmer & Sakagami	1	1	2
	<i>Dialictus</i>	<i>Lasioglossum ephialtum</i> Gibbs	3		3
		<i>Lasioglossum laevissimum</i> (Smith)	1		1
		<i>Lasioglossum novascotiae</i> (Mitchell)	1		1
	<i>Hemihalictus</i>	<i>Lasioglossum inconditum</i> (Cockerell)	3		3
Colletidae	-	<i>Colletes consors</i> Cresson		1	1
	-	<i>Colletes nigrifrons</i> Titus	1	10	11

match. *Osmia nigrifrons* is a metallic *Osmia* whereas *O. nearctica* is a non-metallic species, one of only six non-metallic North American *Osmia* (*Melanosmia*) species, with distinct mandibular and clypeal characteristics that separate it from its closest relatives. We include our identifications in the species records uploaded to BOLD and GenBank.

Discussion

Our surveys contribute to the fundamental knowledge of bee diversity and biogeography in northern Canada, a region that is difficult to survey (e.g., Fig. 2E) and

subject to increasing economic development (Vernier *et al.* 2014). Baseline surveys are necessary for conservation in an era of accelerating climate change. Such surveys assist with monitoring changes in species distributions, including the range expansions and contractions (Casacci *et al.* 2013) that are expected with climate change (Dew *et al.* 2019). Ongoing and future entomological studies in these regions should continue to engage with and seek leadership from indigenous communities to complement environmental stewardship and conservation, and to ensure knowledge is generated, shared, and curated locally.

Our surveys underscore the complementarity (and necessity) of morphological taxonomy when DNA barcoding is used in species-level identifications (Packer *et al.* 2009). DNA barcoding is valuable for insects that may be indistinguishable from one another at particular life stages (e.g., larvae, pupae) and for rapidly assessing biological communities

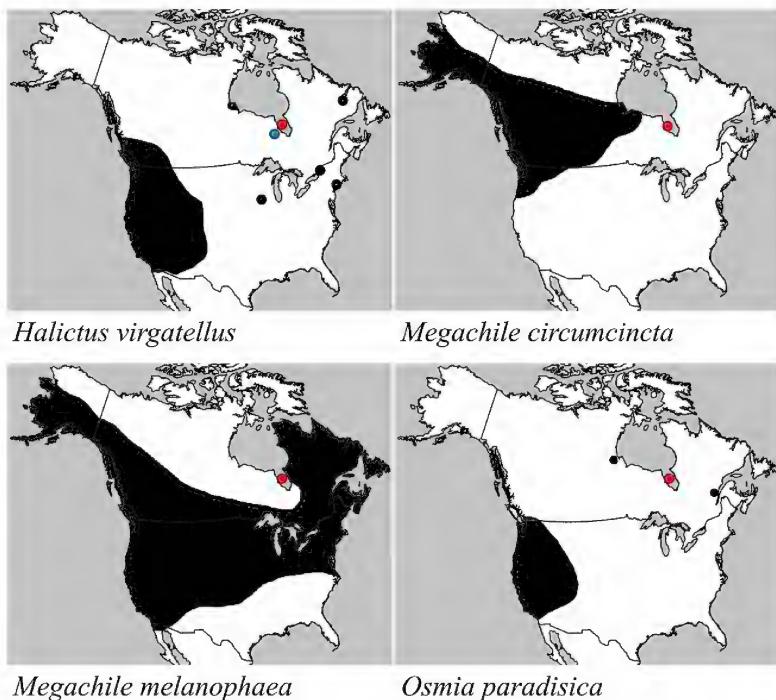


FIGURE 3. New records determined for Nunavut (in red). Two records of *Halictus virgatellus* from our surveys in Northern Ontario are indicated by the blue circle. The dark shading region represents the previous known geographic range of each species, the black circles are locations of individual records. Ranges were determined from maps and records provided in Table S3.

in poorly studied regions (Sheffield *et al.* 2017). However, researchers must not rely solely on DNA barcoding, as existing catalogues may be incomplete (as was the case for *O. nearctica*) or samples may lack DNA of sufficient quality needed for BIN assignment.

A noteworthy record from our survey was a bilateral gynandromorph individual of *M. melanophaea* (Fig. 4). A gynandromorph is an individual of an otherwise non-hermaphroditic species that exhibits both male and female characteristics; bilateral forms, in which one side of the sagittal plane is entirely female and the other side is entirely male, are the rarest examples of gynandromorphism reported in bees (Michez *et al.* 2009). Gynandromorphism is well documented in bees, whose haplodiploid sex determination may facilitate the more frequent production of gynandromorphs (Michez *et al.* 2009). *Megachile* species are disproportionately represented among the documented cases of gynandromorphs (Michez *et al.* 2009), but to our knowledge, this specimen represents the first reported case for *M. melanophaea*.



FIGURE 4. *Megachile melanophaea* bilateral gynandromorph discovered on Akimiski Island, Nunavut (all photos by Liam Graham): (A) frontal view of the head showing dense whitish clypeal hairs and an enlarged, modified foretibial structure on the specimen's left (male) side (right side of the image); (B) metasoma illustrating dense patch of scopal hairs on the penultimate exposed sternite on the specimen's right (female) side (left side of the image); (C) habitus, female side, lateral view; (D) habitus, male side, lateral view.

Among the new territorial records, three (*H. virgatellus*, *M. circumcincta*, and *O. paradisica*) are of biogeographic interest because they are primarily restricted to the western half of the continent and known from only a few records east of Manitoba. In the case of *O. paradisica*, there is a single additional locality in Quebec (BOLD 2020; Sequence ID: BECD371-09) and for *H. virgatellus*, a single preserved specimen from southern Ontario collected in 1978 and curated at Texas A&M University, College Station, Texas, USA (<https://www.gbif.org/occurrence/3048741525>) as well as a single record identified from Labrador within Newfoundland and Labrador (Hicks and Sheffield 2021). And so, our records for these three species are $\geq 1,000$ km east of their primary known ranges. Why are these species found at James Bay or Akimiski Island, which emerged by rebounding from under Pleistocene ice sheets $<5,000$ years ago? This curious disjunct distribution is also exhibited by the horse fly *Hybomitra osburni* (Hine) (Diptera, Tabanidae) (Ringrose *et al.* 2014) and the hover flies *Platycheirus kelloggi* Snow (Diptera, Syrphidae) and *Platycheirus latitarsis* Vockeroth (Diptera, Syrphidae) (Vezsenyi *et al.* 2019). Since the northern regions of eastern and central Canada have been so poorly surveyed, it is possible that these insect species are more widespread in North America than previously thought. Alternatively, truly disjunct distributions may be the result of a combination of glacial retreat and the logging and European agricultural practices that took place beginning in the 1600s, especially in eastern and central Canada. Both processes have separated western and eastern habitats in Canada and severed corridors that connected them (Johnson and Miyanishi 2012; Pendleton *et al.* 2019). As more remote areas are surveyed, other small and easily overlooked species known primarily from west of the Rocky Mountains may be found in more easterly habitats. For example, *Bombus melanopygus* Nylander (Hymeoptera, Apidae), a bumble bee seldom collected east of Manitoba over the last half-century (Williams *et al.* 2014), was also recently found on Akimiski Island and northern Ontario (Gibson *et al.* 2018).

Climate change is projected to drive warmer winters and longer growing seasons in the continental sections of northern Ontario, Nunavut, and much of the rest of boreal Canada (McCabe and Wolock 2010). This could lead to concomitant increases in floral abundance and diversity, and hence, bee abundance and diversity. In contrast, climate change may negatively impact bees in northern ecosystems via several mechanisms. Some flowering plant species that are narrowly adapted to current conditions may decline, driving losses of their associated pollinators (Biesmeijer *et al.* 2006). Decoupled phenological shifts in plants and pollinators (e.g., emergence and blooming times) may lead to temporal mismatches in their interactions (Gérard *et al.* 2020). Baseline surveys such as ours permit comparisons needed to assess the effects of climate change over time and are lacking for insects in northern regions around the world (Høye 2020; Gillespie *et al.* 2020).

Although much of the FNBP and Akimiski Island surveys were focused on Diptera, we capitalized on the bees present in the bulk sample to increase our understanding of their biogeography. Our study demonstrates the important ecological and biogeographical information retrievable from maintained insect by-catch collections (e.g., Hung *et al.* 2015), especially from the world's difficult-to-access regions (Spears and Ramirez 2015; Drinkwater *et al.* 2019).

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APPENDIX 1. Supplementary tables.

TABLE S1: Traps are net (aerial netting), bot (carriion baited bottle trap, LeGros and Beresford 2010), Mal (Malaise trap), Nzi (biting fly Nzi trap), pit (pitfall trap), pan (Y yellow; B blue; W white). Sorted by family, species, Nunavut or Ontario, and latitude.

Species	Unique ID	Genbank	Sex	Trap	Pr./Terr.	Lat. N	Long. W	Date	Month	Year
<i>Andrena algida</i>	1067301B-2012-009-00004	MW339802	F	net	Ont.	52°46'26"	81°57'28"	6	June	2012
<i>Andrena barbilabris</i>	SRP-2014-00340Y-00001	MW339803	F	pan Y	Ont.	56°41'09"	89°14'02"	3	July	2014
<i>Andrena frigida</i>	1067301B-2012-001-00009	MW339801	F	net	Ont.	52°46'26"	81°57'28"	5	June	2012
<i>Anthophora terminalis</i>	AKCP-2015-04	MW340058	M	Nzi	Nvt.	53°06'18"	80°57'23"	19	July	2015
<i>Colletes cunicularius</i>	AKSS-2011-01	Discarded	F	net	Nvt.	52°43'13"	80°59'00"	12	July	2011
<i>Colletes nigritrons</i>	AKBS-2012-07	MW339944	F	pit	Nvt.	53°04'02"	80°58'14"	21-22	July	2012
<i>Colletes nigritrons</i>	AKBS-2012-02	MW339947	F	pit	Nvt.	53°04'02"	80°58'14"	20	July	2012
<i>Colletes nigritrons</i>	AKBS-2012-01	MW339946	F	pit	Nvt.	53°04'02"	80°58'14"	19	July	2012
<i>Colletes nigritrons</i>	AKBS-2012-11	MW339945	F	pit	Nvt.	53°04'02"	80°58'14"	24	July	2012
<i>Colletes nigritrons</i>	AKBS-2012-09	Discarded	F	pit	Nvt.	53°04'02"	80°58'14"	21-22	July	2012
<i>Colletes nigritrons</i>	AKBS-2012-08	Discarded	F	pit	Nvt.	53°04'02"	80°58'14"	21-22	July	2012
<i>Colletes nigritrons</i>	AKBS-2012-04	Discarded	F	pit	Nvt.	53°04'02"	80°58'14"	20	July	2012
<i>Colletes nigritrons</i>	AKBS-2012-03	Discarded	F	pit	Nvt.	53°04'02"	80°58'14"	20	July	2012
<i>Colletes nigritrons</i>	AKCP-2013-01	MW339943	F	pit	Nvt.	53°06'18"	80°57'25"	20	July	2013
<i>Colletes nigritrons</i>	AKCP-2011-01	Discarded	F	pit	Nvt.	53°06'18"	80°57'25"	26	July	2011
<i>Colletes nigritrons</i>	DEG-2014-00073B-00014	MW339979	M	pan Y	Ont.	56°34'04"	89°24'23"	4	July	2012
<i>Halictus confusus</i>	AS-2015-00054-00003	Discarded	F	pan B	Ont.	51°50'25"	83°00'31"	4	July	2012
<i>Halictus rubricundus</i>	1087961B-2012-085-00003	MW339957	F	net	Ont.	51°58'07"	82°20'23"	22	June	2012
<i>Halictus rubricundus</i>	1087961B-2012-069-00001	MW339956	F	net	Ont.	51°58'07"	82°20'23"	22	June	2012
<i>Halictus virginellus</i>	AKBS-2013-12	MW339968	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2013-11	MW339967	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2012-10	MW339978	-	pit	Nvt.	53°04'02"	80°58'14"	24	July	2012
<i>Halictus virginellus</i>	AKBS-2013-01	MW339954	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2013-10	MW339959	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2013-09	MW339949	F	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2013-08	MW339950	F	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2013-07	MW339951	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2013-05	MW339952	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2013-03	MW339953	F	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halictus virginellus</i>	AKBS-2012-06	Discarded	F	pit	Nvt.	53°04'02"	80°58'14"	20	July	2012
<i>Halictus virginellus</i>	AKBS-2013-06	MW339948	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013

TABLE S1 continued...

Species	Unique ID	Genbank	Sex	Trap	Pr./Terr.	Lat.N	Long.W	Date	Month	Year
<i>Halicitus virgatellus</i>	AKBS-2013-15	Discarded	M	pit	Nvt.	53°04'02"	80°58'14"	29	July	2013
<i>Halicitus virgatellus</i>	AKCP-2018-04	Discarded	M	Nzi	Nvt.	53°06'18"	80°57'25"	23	August	2009
<i>Halicitus virgatellus</i>	DEG-2014-0006W-00016	Discarded	F	pan W	Ont.	56°34'04"	89°24'25"	5	July	2014
<i>Halicitus virgatellus</i>	DEG-2014-00062B-00022	Discarded	F	pan Y	Ont.	56°34'04"	89°24'25"	3	July	2014
<i>Halicitus virgatellus</i>	DEG-2014-00062B-00022	Discarded	F	pit	Nvt.	53°06'18"	80°57'25"	20	July	2012
<i>Lasioglossum boreale</i>	AKBS-2012-13	Discarded	F	net	Ont.	52°18'23"	84°36'57"	11	July	2012
<i>Lasioglossum boreale</i>	1101691B-2012-012-00009	MW339990	F	Mal	Ont.	51°55'45"	83°20'44"	7	June	2012
<i>Lasioglossum ephialtum</i>	1101691B-2012-046-00005	Discarded	F	Nzi	Ont.	52°18'23"	84°36'57"	13	June	2012
<i>Lasioglossum ephialtum</i>	1101691B-2012-101-00003	MW339976	F	Nzi	Ont.	52°18'23"	84°36'57"	14	July	2014
<i>Lasioglossum ephialtum</i>	1101691B-2012-101-00003	MW340059	M	Mal	Ont.	51°55'45"	83°20'44"	6	July	2012
<i>Lasioglossum inconditum</i>	1101706B-2012-014-00015	MW339986	F	Mal	Ont.	51°55'45"	83°20'44"	6	July	2012
<i>Lasioglossum inconditum</i>	1101706B-2012-014-00013	MW339987	F	Nzi	Ont.	52°18'23"	84°36'57"	14	July	2012
<i>Lasioglossum inconditum</i>	1101691B-2012-101-00004	MW339988	F	Mal	Ont.	52°18'23"	84°36'57"	13	June	2012
<i>Lasioglossum laevissimum</i>	1101691B-2012-066-00031	MW339977	F	Mal	Ont.	51°55'45"	83°20'44"	7	June	2012
<i>Lasioglossum novasociae</i>	1101706B-2012-046-00010	Discarded	F	Mal	Ont.	51°55'45"	83°20'44"	7	June	2012
Megachilidae										
<i>Coeilioxys sodalis</i>	AKCP-2008-02	MW340004	M	net	Nvt.	53°06'18"	80°57'25"	1	August	2008
<i>Coeilioxys sodalis</i>	AS-2015-00054-00001	MW339940	M	pan Y	Ont.	51°50'25"	83°00'31"	4	July	2012
<i>Hoplitis albibrons</i>	AS-2015-00056-00001	MW340005	M	pan Y	Ont.	52°38'11"	82°11'10"	4	July	2012
<i>Hoplitis albibrons</i>	1074717B-2012-064-00002	MW339964	M	net	Ont.	52°53'33"	82°41'25"	23	June	2012
<i>Hoplitis albibrons</i>	1074717B-2012-064-00010	MW339965	M	net	Ont.	54°09'45"	93°57'23"	12	June	2011
<i>Megachile circumcincta</i>	AKCP-2017-24	MW339896	F	Nzi	Nvt.	53°06'18"	80°57'25"	17	July	2017
<i>Megachile frigida</i>	AKCP-2015-08	MW339999	F	Mal	Nvt.	53°06'18"	80°57'25"	20-24	July	2015
<i>Megachile frigida</i>	AKCP-2015-02	MW340000	F	Nzi	Nvt.	53°06'18"	80°57'25"	19	July	2015
<i>Megachile frigida</i>	1074717B-2012-079-00013	MW339997	M	net	Ont.	52°33'33"	83°18'57"	24	June	2012
<i>Megachile frigida</i>	1074717B-2012-066-00001	MW339996	M	Nzi	Ont.	52°33'33"	83°18'57"	23	June	2012
<i>Megachile gemmata</i>	AKCP-2013-03	MW340049	F	net	Nvt.	53°06'18"	80°57'25"	30	July	2013
<i>Megachile gemmata</i>	AKCP-2015-01	MW340001	F	Nzi	Nvt.	53°06'18"	80°57'25"	19	July	2015
<i>Megachile gemmata</i>	KGD-2013-00075-00001	Discarded	F	pit	Ont.	51°03'40"	87°37'57"	14	July	2013
<i>Megachile laponica</i>	AKCP-2012-01	MW339994	M	Mal	Nvt.	53°06'18"	80°57'25"	23	July	2012
<i>Megachile laponica</i>	AKCP-2015-03	MW339995	F	Nzi	Nvt.	53°06'18"	80°57'25"	19	July	2015
<i>Megachile laponica</i>	DEG-2013-00090-00001	MW339991	M	net	Ont.	51°00'40"	88°51'06"	2	July	2013
<i>Megachile laponica</i>	DEG-2013-00134-00001	MW340003	F	Nzi	Ont.	51°00'40"	88°51'06"	4	July	2013
<i>Megachile laponica</i>	1081060B-2012-109-00001	Discarded	-	bot	Ont.	52°23'36"	83°51'58"	8	July	2012
<i>Megachile melanophaea</i>	AKBC-2011-01	MW340043	F	pit	Nvt.	53°04'18"	80°38'14"	27	July	2011
<i>Megachile melanophaea</i>	AKCP-2017-12	MW340043	F	Nzi	Nvt.	53°06'18"	80°57'25"	19	July	2017
<i>Megachile melanophaea</i>	AKCP-2017-20	MW340054	syn.M	net	Nvt.	53°06'18"	80°57'25"	25-26	July	2017
<i>Megachile perihirta</i>	AKCP-2013-04	MW339992	M	Mal	Nvt.	53°06'18"	80°57'25"	30	July	2013
<i>Megachile perihirta</i>	AKCP-2013-02	MW340008	F	Nzi	Nvt.	53°06'18"	80°57'25"	18	July	2013
<i>Megachile perihirta</i>	AKCP-2015-07	MW339993	F	Nzi	Nvt.	53°06'18"	80°57'25"	28	July	2015

TABLE S1 continued...

Species	Unique ID	Genbank	Sex	Trap	Pr./Terr.	Lat. N	Long. W	Date	Month	Year
<i>Megachile relativa</i>	AKCP-2015-05	MW340007	F	Mal	Nvt.	53°06'18"	80°57'23"	21	July	2015
<i>Osmia bucephala</i>	AKCP-2017-09	Discarded	F	Nzi	Nvt.	53°06'18"	80°57'25"	18	July	2017
<i>Osmia bucephala</i>	II0169IB-2012-012-00008	Discarded	F	net	Ont.	52°18'23"	84°36'57"	11	July	2012
<i>Osmia laticeps</i>	SVL-2010-001-34-00001	Discarded	F	net	Ont.	52°36'58"	80°05'17"	22	June	2010
<i>Osmia neearctica</i>	II01706B-2012-001-00001	Discarded	F	net	Ont.	51°55'45"	83°20'44"	5	June	2012
<i>Osmia neearctica</i>	II07417IB-2012-064-00003	MW34044	F	net	Ont.	52°53'33"	83°18'57"	23	June	2012
<i>Osmia neearctica</i>	ILR-2013-00178-00001	MW34046	F	net	Ont.	53°23'46"	87°48'09"	27	June	2013
<i>Osmia neearctica</i>	AKCP-2017-16	MW34048	F	Mal	Nvt.	53°06'18"	80°57'25"	22-23	July	2017
<i>Osmia neearctica</i>	AKCP-2017-11	Discarded	F	Nzi	Nvt.	53°06'18"	80°57'25"	19	July	2017
<i>DEG-2013-00135B-00003</i>	MW34040	F	pan B	Ont.	51°00'40"	88°51'06"	4	July	2013	
<i>Osmia nigroviridis</i>	II15456B-2012-069-00001	MW340038	F	pit	Ont.	51°47'49"	84°57'48"	7	July	2012
<i>Osmia nigroviridis</i>	II15456B-2012-014-00001	MW34047	F	pit	Ont.	51°47'49"	84°57'35"	5	July	2012
<i>Osmia nigroviridis</i>	106730IB-2012-009-00002	MW340041	M	net	Ont.	52°46'27"	82°02'31"	6	June	2012
<i>Osmia nigroviridis</i>	106730IB-2012-001-00008	MW340039	M	net	Ont.	52°46'27"	81°57'29"	5	June	2012
<i>Osmia nigroviridis</i>	AKCP-2015-06	MW340055	F	Nzi	Nvt.	53°06'18"	80°57'25"	25	July	2015
<i>Osmia nigroviridis</i>	AKCP-2017-19	MW339871	F	Nzi	Nvt.	53°06'18"	80°57'25"	23-24	July	2017
<i>Osmia nigroviridis</i>	AKCP-2017-13	MW340042	F	Nzi	Nvt.	53°06'18"	80°57'25"	19-20	July	2017
<i>Osmia nigroviridis</i>	AKCP-2017-08	Discarded	F	Nzi	Nvt.	53°06'18"	80°57'25"	18	July	2017
<i>Osmia nigroviridis</i>	AKCP-2017-01	MW340045	F	Nzi	Nvt.	53°06'18"	80°57'25"	16	July	2017
<i>Osmia nigroviridis</i>	AKCP-2012-05	MW340050	F	pit	Nvt.	53°06'18"	80°57'25"	22	July	2012
<i>Osmia paradisica</i>	AKCP-2012-04	MW340051	F	pit	Nvt.	53°06'18"	80°57'25"	21	July	2012
<i>Osmia paradisica</i>	AKCP-2012-03	MW340052	F	pit	Nvt.	53°06'18"	80°57'25"	21	July	2012
<i>Osmia paradisica</i>	AS-2015-00060-00002	MW340053	F	net	Ont.	52°03'42"	82°47'33"	14	July	2012
<i>Osmia tarsata</i>	TRI-2014-00027TW-00001	MW340057	F	pan W	Ont.	54°45'04"	90°38'17"	20	June	2014
<i>Osmia tarsata</i>										

TABLE S2. List of some life history traits for each species identified in the survey.

Family	Subgenus	Species	Pollen specialization	Sociality	Nesting preference
Apidae	<i>Clisodon</i>	<i>Anthophora terminalis</i> Cresson	Polylectic ¹	Solitary ¹⁰	Wood, stems ²
	<i>Coelioxys</i>	<i>Coelioxys sodalis</i> Cresson	Cleptoparasitic ²		Cleptoparasite ²
Megachilidae	<i>Alcidamea</i>	<i>Hoplitis albifrons</i> (Kirby)	Polylectic ²	Solitary ¹⁰	Pre-existing cavity ¹⁶
	<i>Megachile</i>	<i>Megachile laponica</i> Thomson	Polylectic ¹	Solitary ¹⁰	Pre-existing cavity ²
		<i>Megachile relativa</i> Cresson	Polylectic ¹	Solitary ¹⁰	Pre-existing cavity ²
		<i>Megachile circumcincta</i> (Kirby)	Polylectic ²	Solitary ¹⁰	Soil ²
		<i>Megachile ffigida</i> Smith	Polylectic ¹	Solitary ¹⁰	Pre-existing cavity ²
		<i>Megachile gemula</i> Cresson	Polylectic ¹	Solitary ¹⁰	Pre-existing cavity ²
		<i>Megachile melanophoea</i> Smith	Polylectic ¹	Solitary ¹⁰	Soil ²
		<i>Megachile perihirta</i> Cockerell	Oligolectic (Asteraceae) ²	Solitary ¹⁰	Pre-existing cavity ²
		<i>Osmia bicolorata</i> Cresson	Polylectic ¹	Solitary ¹⁰	Pre-existing cavity ²
		<i>Osmia laticeps</i> Thomson	Oligolectic (Ericaceae) ³	Solitary ¹⁰	Pre-existing cavity ¹⁷
		<i>Osmia nearctica</i> Rightmyer, Griswold and Arduser	Unknown	Solitary ¹⁰	Unknown
		<i>Osmia nigrovittata</i> (Zeiterstedt)	Polylectic ³	Solitary ¹⁰	Pre-existing cavity ³
		<i>Osmia paradoxa</i> Sandhouse	Unknown	Solitary ¹⁰	Unknown
		<i>Osmia tarsata</i> Provancher	Unknown	Solitary ¹⁰	Soil ¹⁸
		<i>Osmia tersella</i> Cockerell	Polylectic ¹	Solitary ¹⁰	Pre-existing cavity ²
Andrenidae	<i>Andrena</i>	<i>Andrena ffigida</i> Smith	Oligolectic (<i>Salix</i>) ¹	Solitary ¹⁰	Soil ²
	<i>Euanadrena</i>	<i>Andrena agilda</i> Smith	Polylectic ¹	Solitary ¹⁰	Soil ²
	<i>Leucandrena</i>	<i>Andrena barbilabris</i> (Kirby)	Polylectic ⁴	Solitary ¹⁰	Soil ²
	<i>Protohalictus</i>	<i>Halticus rubicundus</i> (Christ)	Polylectic ²	Variable ²	Soil ²
	<i>Seladonia</i>	<i>Halticus confusus</i> Smith	Polylectic ⁵	Variable ² ; eusocial in southern Ontario ¹¹	Soil ¹¹
		<i>Halticus virgatellus</i> Cockerell	Polylectic ²	Variable; eusocial in southern Ontario ¹¹	
				range ^{2,12}	
				Solitary ¹³	
				Eusocial ¹⁴	
Halictidae	<i>Dialictus</i>	<i>LasioGLOSSUM epiphilum</i> Gibbs	Polylectic ¹		Soil ¹³
		<i>LasioGLOSSUM laevissimum</i> (Smith)	Polylectic ¹		Soil ¹⁴
		<i>LasioGLOSSUM novascotiae</i> (Mitchell)	Polylectic ⁷		Soil ²
	<i>Hemihalictus</i>	<i>LasioGLOSSUM inconditum</i> (Cockerell)	Polylectic ¹		Soil ⁷
				Soil ²	

TABLE S2 continued...

Family	Subgenus	Species	Pollen specialization	Sociality	Nesting preference
Coletididae	-	<i>Colletes cossorus</i> Cresson	Polylectic ^{a,9}	Solitary ¹⁰	Soil ⁸
	-	<i>Colletes nigritrons</i> Titus	Polylectic ²	Solitary ¹⁰	Soil ²

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² Sheffield, C.S., Frier, S.D., and Dumesch, S. 2014. The bees (Hymenoptera: Apoidea, Apiformes) of the Prairies Ecozone with comparisons to other grasslands of Canada. Pp. 427–467 In Gibsonson, D.J. and Cárcamo, H.A. (eds). Arthropods of Canadian Grasslands (Volume 4). Biodiversity and Systematics Part 2. Biological Survey of Canada, Ottawa, Ontario, Canada.

³ Rightmyer, M.G., Griswold, T. and Arduser, M.S. 2010. A review of the non-metallic *Osmia* (*Melanosmia*) found in North America, with additional notes on palearctic *Melanosmia* (Hymenoptera, Megachilidae). *ZooKeys*, **60**: 37–77. doi: 10.3897/zookeys.60.484.

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⁶ Andreas, P., Ebner, W., Svensson, B.G., and Sakagami, S.F. 1977. *LasioGLOSSUM (Eylaeus) boreale*, a new Halictinae (Hymenoptera: Apoidea) species found in Northern Sweden and on Hokkaido, Japan, with notes on its biology. *Insect Systematics and Evolution*, **8**: 219–229. doi: 10.1163/18761277X00297.

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⁹ Onufreko, T.M. and Hutchings, G.E. 2021. Discovery and description of the holarctic first instar of *Epeorus americanus* (Cresson) (Hymenoptera: Apidae), a cleoparasite of *Colletes cossorus mesocopus* Swenk (Hymenoptera: Colletidae). *Journal of the Entomological Society of Ontario*, **152**: 1–13.

¹⁰ Michener, C.D. 2007. The Bees of the World, 2nd ed. Johns Hopkins University Press, Baltimore, Maryland.

¹¹ Richards, M.H., Vickruck, J.L., and Rehan, S.M. 2010. Colony social organisation of *Halicitus confusus* in southern Ontario, with comments on sociality in the subgenus *H.* (*Seladonia*). *Journal of Hymenoptera Research*, **19**: 144–158.

¹² Hicks, B. and Sheffield, C. 2021. Native bees (Hymenoptera: Apoidea) collected from Labrador, Canada. *Journal of the Acadian Entomological Society*, **17**: 20–24.

¹³ Packer, L. and Taylor, J.S. 2002. Genetic variation within and among populations of an arctic/alpine sweat bee (Hymenoptera: Halictidae). *The Canadian Entomologist*, **134**: 619–631. doi: 10.4039/Ent134619.5.

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¹⁷ Rust, R.W., Thorp, R.W., and Forchio, P.E. 1974. The ecology of *Osmia nigritrons* with a comparison to other *Xanthomioidae*. *Journal of Natural History*, **8**: 29–47. doi: 10.1080/00222937.400700041.

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TABLE S3. The provincial and territorial distribution of the bee species identified in our survey. An * indicates a new territorial record for Nunavut.

Family	Subgenus	Species	Species Recorded From	Literature Reference(s)
Apidae	<i>Clisodon</i>	<i>Anthophora terminalis</i> Cresson	YT + NWT + BC + AB + SK + MB + QC (CNC, GBIF, NatureServe), ON (CNC, Harvard Museum of Comparative Zoology, GBIF, NatureServe), NB + NS (NatureServe)	ON + QC (Satyshur and Ort, 2020), NS (Sheffield et al. 2013), BC (Sheffield and Heron 2019)
Megachilidae	<i>Coelioxys</i>	<i>Coelioxys sodalis</i> Cresson	YT + NWT + BC (CNC, GBIF, NatureServe), AB + SK + MB + ON + QC + NB + NS + PEI (CNC, 2012).	All Canadian provinces and Territories (De Silva and Natureserve)
Megachilidae	<i>Alcidamea</i>	<i>Hoplitis abifrons</i> (Kirby)	YT + NWT + NU + BC + AB + SK + MB (GBIF Natureserve), ON (CNC, GBIF, NatureServe), NL + NB + NS (NatureServe)	YT (Leung and Forrest 2019), BC (Sheffield and Heron 2019), AB + SK + MB (Sheffield, Frier and Dunesh 2014)
Megachilidae	<i>Megachile</i>	<i>Megachile lapponica</i> Thomson	NWT + AB + ON (CNC, GBIF, NatureServe), YT + NU + SK + QC + NL + NB + NS (NatureServe)	BC (Sheffield and Heron 2019; Sheffield et al. 2011), AB + SK + MB (Sheffield, Frier and Dunesh 2014; Sheffield et al. 2011), YT + NWT + ON + QC + NB (Sheffield et al. 2011)
Megachilidae	<i>Megachile</i>	<i>Megachile relativa</i> Cresson	ON (CNC, Harvard Museum of Comparative Zoology, GBIF, NatureServe), QC (CNC, GBIF, NatureServe), YT + NWT + NU + BC + AB + SK + MB + NL + NB + NS + PEI (GBIF, NatureServe)	BC (Sheffield and Heron 2019; Sheffield et al. 2011), AB + SK + MB (Sheffield, Frier and Dunesh 2014; Sheffield et al. 2011), YT + NWT + NU + NS (Sheffield et al. 2013; Sheffield et al. 2011), YT + NWT + ON + QC + NB (Sheffield et al. 2011)
Megachilidae	<i>Xanthosarus</i>	<i>Megachile circumcincta</i> (Kirby)*	YT + NWT + AB (GBIF, NatureServe), BC + SK + MB (NatureServe)	BC (Sheffield and Heron 2019), YT + NWT + NU + AB + SK + MB (Sheffield et al. 2011)
Megachilidae	<i>Xanthosarus</i>	<i>Megachile frigida</i> Smith	NU (GBIF), YT + NWT + BC + AB + MB + ON + QC + NL (CNC, GBIF, NatureServe), NB + NS + PEI (NatureServe)	BC (Sheffield et al. 2013; Sheffield et al. 2011), YT + NWT + AB + SK + MB + ON + QC + NB + NS + PEI + NL (Sheffield et al. 2011), BC (Sheffield and Heron 2019; Sheffield et al. 2011), YT + NWT + NU + AB + SK + MB + ON + QC + NB (Sheffield et al. 2013; Sheffield et al. 2011)
Megachilidae	<i>Xanthosarus</i>	<i>Megachile gemula</i> Cresson	NWT (GBIF, NatureServe), BC + AB + SK + ON + QC + NB + NS (CNC, GBIF, NatureServe)	BC (Sheffield and Heron 2019; Sheffield et al. 2011), YT + NWT + NU + AB + SK + MB + ON + QC + NB (Sheffield et al. 2013; Sheffield et al. 2011)
Megachilidae	<i>Xanthosarus</i>	<i>Megachile melanophaea</i> Smith*	YT + NWT + BC + AB + SK + MB + ON + QC (CNC, GBIF, NatureServe), NL + NB + NS + PEI (GBIF, NatureServe)	BC (Sheffield and Heron 2019; Sheffield et al. 2011), AB + SK + MB (Sheffield, Frier and Dunesh 2014; Sheffield et al. 2011), YT + NWT + ON + QC + NB + NS + PEI + NL (Sheffield et al. 2011)

TABLE S3 continued..

Family	Subgenus	Species	Species Recorded From	Literature Reference(s)
Megachilidae	Xanthosaris	<i>Megachile peritincta</i> Cockerell	NWT + BC + MB (CNC, GBIF, Natureserve), ON (CNC, GBIF, Natureserve), SK (Natureserve)	BC (Sheffield and Heron 2019; Sheffield <i>et al.</i> 2011), AB + SK + MB (Sheffield, Frier and Dumesh 2014; Sheffield <i>et al.</i> 2011), YT + NWT + ON (Sheffield <i>et al.</i> 2011)
Megachilidae	<i>Melanosmia</i>	<i>Osmia bucephala</i> Cresson	ON (Harvard Museum of Comparative Zoology, GBIF, Natureserve), QC (CNC, GBIF, Natureserve), YT + NWT + NU + BC + AB + SK + MB + NL + NB + NS + PEI (GBIF, Natureserve)	BC (Sheffield and Heron 2019), AB (Sheffield, Frier and Dumesh, 2014), NS (Sheffield <i>et al.</i> 2013)
Megachilidae	<i>Melanosmia</i>	<i>Osmia laticeps</i> Thomson	YT + NU + MB + ON + QC + NB + NS (GBIF, Natureserve)	YT + NWT + NU + BC + AB + SK + MB ON + QC + NB + NS (Rightmyer <i>et al.</i> 2010)
Megachilidae	<i>Melanosmia</i>	<i>Osmia necreitica</i> Rightmyer; Griswold and Arduser	YT + NWT (CNC, GBIF, Natureserve), NU + MB + ON + QC (GBIF, Natureserve)	YT + NWT + NU + ON + QC (Rightmyer <i>et al.</i> 2010)
Megachilidae	<i>Melanosmia</i>	<i>Osmia nigrovittata</i> (Zetterstedt)	YT + NWT + BC + AB + MB + QC + NL + NB + NS + PEI (GBIF, Natureserve), ON (CNC)	YT + NWT + NU + BC + AB + SK + MB ON + QC + NB + NS + PEI + NL (Rightmyer <i>et al.</i> 2010)
Megachilidae	<i>Melanosmia</i>	<i>Osmia paradisiaca</i> Sandhouse*	YT + BC + MN + QC (CNC, Natureserve, GBIF)	BC (Sheffield and Heron 2019)
Megachilidae	<i>Melanosmia</i>	<i>Osmia tarsata</i> Provancher	ON (GBIF, Natureserve), YT + AB + QC + NL + NB + NS + PEI (Natureserve)	QC (Moisan-DeSerres <i>et al.</i> 2014)
Megachilidae	<i>Melanosmia</i>	<i>Osmia terpsila</i> Cockerell	All Canadian Provinces and Territories (CNC, GBIF, Natureserve)	QC (Moisan-DeSerres <i>et al.</i> 2014), NS (Sheffield <i>et al.</i> 2013)
Andrenidae	<i>Andrena</i>	<i>Andrena frigida</i> Smith	YT + NWT (Natureserve), BC + AB + SK (Natureserve, GBIF), ON (CNC, Harvard Museum of Comparative Zoology, GBIF, Natureserve), MB + QC (CNC, GBIF, Natureserve), NL + NS + NB + PEI (Natureserve, GBIF)	BC (Sheffield and Heron 2019), AB + SK + MB (Sheffield, Frier and Dumesh 2014), NB (Ostaff <i>et al.</i> 2015)
Andrenidae	<i>Euanthrena</i>	<i>Andrena algida</i> Smith	YT + NWT + BC + AB (CNC, GBIF), SK + MB + ON + QC (CNC, GBIF Natureserve), NB + NS + PEI (Natureserve)	AB + SK + MB (Sheffield, Frier and Dumesh 2014), QC (Moisan-DeSerres <i>et al.</i> 2014), NS (Sheffield <i>et al.</i> 2013)
Andrenidae	<i>Leucandrena</i>	<i>Andrena barbilabris</i> Kirby	YT + NWT (GBIF, Natureserve), BC + AB + SK + MB + ON + QC (CNC, GBIF, Natureserve), NL + NB + NS + PEI (Natureserve)	BC (Sheffield and Heron 2019), AB + SK + MB (Sheffield, Frier and Dumesh 2014), NS (Sheffield <i>et al.</i> 2013)
Halticidae	<i>Protohalictus</i>	<i>Halticus rubicundus</i> (Christi)	ON (CNC, Harvard Museum of Comparative Zoology, GBIF, Natureserve), YT + NWT + NU + BC + SK + ON + QC + NL + NB + NS + PEI (GBIF, Natureserve), AB (GBIF)	AB + SK + MB (Sheffield, Frier and Dumesh 2014), ON (Richards <i>et al.</i> 2011), QC (Moisan-DeSerres <i>et al.</i> 2014), NS (Sheffield <i>et al.</i> 2013)

TABLE S3 continued...

Family	Subgenus	Species	Species Recorded From	Literature Reference(s)
Halictidae	<i>Seladonia</i>	<i>Halictus confusus</i> Smith	ON (CNC, Harvard Museum of Comparative Zoology, GBIF, NatureServe), YT + NWT + NU + AB (GBIF), BC + SK + MB + QC + NB + NS + PEI (NatureServe)	BC (Sheffield and Heron 2019) AB + SK + MB (Sheffield, Frier and Dumesh 2014), ON (Richards <i>et al.</i> 2011), QC (Moisan-Desertes <i>et al.</i> 2014), NS (Sheffield <i>et al.</i> 2013)
Halictidae	<i>Seladonia</i>	<i>Halictus virginellus</i> Cockerell*	BC + MB + ON (CNC, GBIF, NatureServe)	BC (Sheffield and Heron 2019), AB + MB (Sheffield, Frier and Dumesh 2014), ON (GBIF (https://doi.org/10.15468/dl.s29efal)), NL (Hicks and Sheffield, 2021)
Halictidae	<i>Sphexodogaster</i>	<i>Lasioglossum boreale</i> Svensson	YT + NWT + BC + MB (NatureServe, GBIF), QC (CNC, NatureServe)	BC (Sheffield and Heron 2019)
Halictidae	<i>Dialictus</i>	<i>Lasioglossum epithalium</i> Gibbs	MB (NatureServe, GBIF), ON (Harvard Museum of Comparative Zoology, GBIF, NatureServe), QC + NB + NS (NatureServe)	ON (Gibbs <i>et al.</i> 2011), Richards <i>et al.</i> (2011), QC (Moisan-Desertes <i>et al.</i> 2014), NS (Moisan-DeSertes <i>et al.</i> 2014)
Halictidae	<i>Dialictus</i>	<i>Lasioglossum laevissimum</i> (Smith)	NWT (NatureServe), BC + SK + MB + ON + NL + NB + NS + PEI (NatureServe, GBIF), AB (GBIF)	BC (Sheffield and Heron 2019), AB + SK (Sheffield, Frier and Dumesh 2014), ON (Richards <i>et al.</i> 2011), QC (Moisan-Desertes <i>et al.</i> 2014)
Halictidae	<i>Dialictus</i>	<i>Lasioglossum novascotiae</i> (Mitchell)	YT + NWT + BC + SK + MB + ON + QC + NB + PEI (NatureServe), NS (GBIF, NatureServe)	BC (Sheffield and Heron 2019), AB (Sheffield, Frier and Dumesh 2014), NS (Sheffield <i>et al.</i> 2013)
Halictidae	<i>Hemihalictus</i>	<i>Lasioglossum inconditionum</i> (Cockerell)	All Canadian provinces and territories (GBIF, NatureServe)	BC (Sheffield and Heron 2019), AB + SK + MB (Sheffield, Frier and Dumesh 2014)
Colletidae	-	<i>Colletes cunicularius</i> Cresson	YT + BC + AB + SK + MB + ON (CNC, GBIF, NatureServe), QC + NB + NS (CNC, NatureServe)	BC (Sheffield and Heron 2019), AB + SK + MB (Sheffield, Frier and Dumesh 2014), QC (Moisan-DeSertes <i>et al.</i> 2014)
Colletidae	-	<i>Colletes nigritrons</i> Titus	AB + SK + QC (CNC, GBIF), ON (GBIF), NWT + NF (NatureServe)	BC (Sheffield and Heron 2019), AB + MB (Sheffield, Frier and Dumesh 2014)

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FIRST RECORD OF THE PLANTHOPPER PARASITE MOTH *FULGORAE CIA EXIGUA* (EDWARDS) (LEPIDOPTERA: EPIPYROPIDAE) IN CANADA, WITH NOTES ON ITS COLLECTION AND HOSTS

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Scientific Note

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Fulgoraecia exigua (Edwards) is a widespread but localized species in the eastern United States occurring from New Jersey and Ohio south to Florida and west to Texas (Covell 1984; Summerville *et al.* 1999), with scattered specimens collected from as far west as Arizona (Edwards 1882). This species seems to be localized in habitat but when encountered can be numerous (JD pers. obs.). It is the sole Nearctic species of Epipyropidae, a family in the superfamily Zygaenoidea that consists of specialist ectoparasites and ectoparasitoids of Auchenorrhyncha and Sternorrhyncha that is most diverse in southeast Asia (Świerczewski *et al.* 2016; Liu *et al.* 2018). Specific information regarding the biology of *F. exigua* is limited, but in other epipyropids the female lays eggs on plants in the vicinity of potential hosts, and host selection is by the active first instar (e.g., Świerczewski *et al.* 2016, Liu *et al.* 2018, Sankararaman *et al.* 2020).

On 7 August 2020, GP photographed an individual of *Acanalonia conica* (Say) with its wings sitting irregularly as a result of a distinct white protuberance on the side of its abdomen, a caterpillar of *Fulgoraecia exigua* with its characteristic waxy coating (Figs. 1–3). After corresponding with SP, it was posted to iNaturalist (<https://www.inaturalist.org/>) (Table 1) and represents the first record of this family within Canada (Pohl *et al.* 2018). On 11–13 August 2020, a targeted survey was made to look for additional specimens in two sites (Ojibway Park and the adjacent Ojibway Prairie Provincial Nature Reserve) of the area generally referred to as the Ojibway Prairie complex (see Paiero *et al.* 2010 for map) in Windsor, Ontario. This area supports a diverse assemblage of insects, including elements of Carolinian forest and grassland habitats, and an effort was made to survey the local Fulgoroidea species for *F. exigua*. Searches focused specifically on detecting

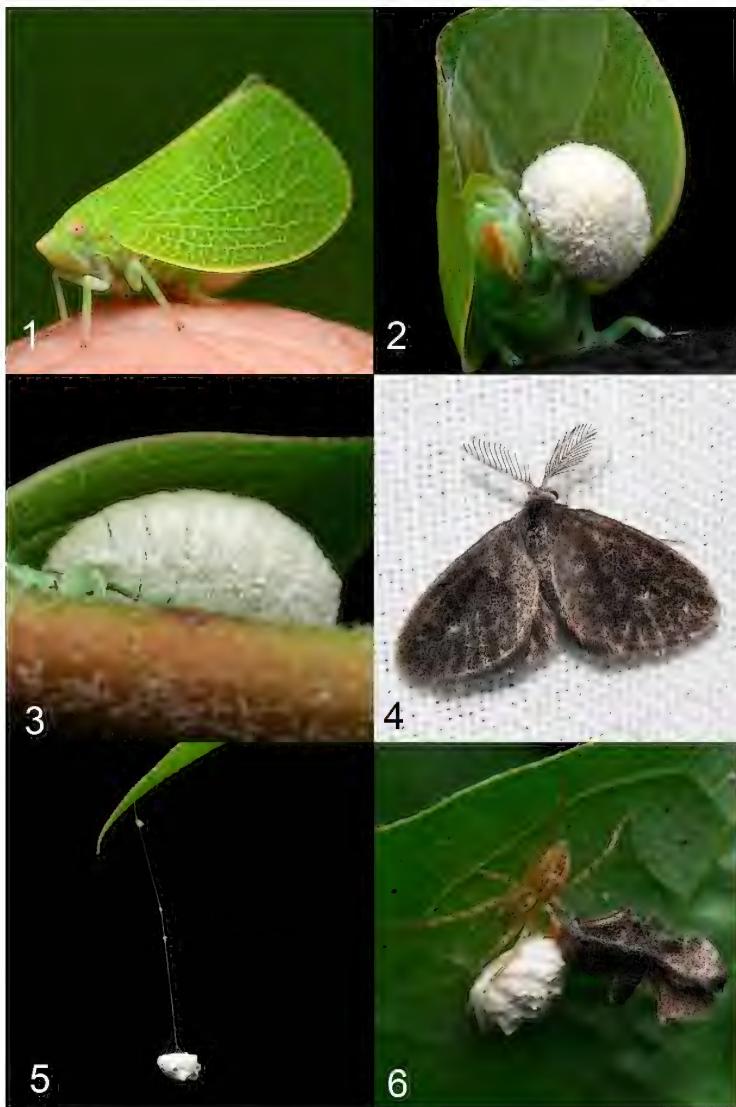
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FIGURES 1–6. *In situ* observations. 1–3, *Acanalonia conica* (Say) with a *Fulgoraecia exigua* (Edwards) caterpillar on the side of its wing, representing the first Canadian record of *F. exigua* and showing the wing ‘dysplasia’ of the host and white waxy coating of the caterpillar. 4, adult male *F. exigua* on a light sheet, representing the first Canadian record of an adult. 5, *F. exigua* pupa hanging on silken thread. 6, *F. exigua* pupa directly attached to a leaf with an unsuccessfully emerged adult of *F. exigua* and a passing spider. Images for Figs 1–3 and 5–6 by G. Pekor and Fig. 4 by J. Vandermeulen.

TABLE 1. Summary of posted iNaturalist observations of *Fulgoraecia exigua* (Edwards) from Ontario in 2020.

Date of Observation	Stage	Observer	iNaturalist Observation
7 August 2020	larva	G. Pekor	https://inaturalist.ca/observations/56085703
22 August 2020	adult	J. Vandermeulen & B. Conlin	https://inaturalist.ca/observations/57591772
26 August 2020	adult	G. Pekor, T. Preney	https://inaturalist.ca/observations/57711888 ; https://inaturalist.ca/observations/57932770
30 August 2020	adult	T. Preney	https://inaturalist.ca/observations/58149000
3 September 2020	adult	T. Preney	https://inaturalist.ca/observations/58519975

TABLE 2. Summary of known Fulgoroidea host species of *Fulgoraecia exigua* (Edwards) from published accounts and online sources. Asterisks (*) denote identifications made by the primary author.

Family	Species	Source
Acanaloniiidae	<i>Acanalonia conica</i> (Say)	https://inaturalist.ca/observations/30990964 ; https://bugguide.net/node/view/427001/bgimage ; https://bugguide.net/node/view/1018279 ; https://bugguide.net/node/view/460831/bgimage ; https://bugguide.net/node/view/532667/bgimage ; https://bugguide.net/node/view/745780/bgimage
	<i>Acanalonia servillei</i> Spinola	https://bugguide.net/node/view/322761/bgimage
	<i>Acanalonia</i> sp. (nymph)	https://bugguide.net/node/view/322761/bgimage
Flatidae	<i>Metcalfa pruinosa</i> (Say)	https://www.inaturalist.org/observations/56990206 ; https://inaturalist.ca/observations/19460809 ; https://bugguide.net/node/view/1875221/bgimage
	<i>Flatormenis proxima</i> (Walker)	https://bugguide.net/node/view/492031/bgimage
Issidae	<i>Aplos simplex</i> (Germar):	https://bugguide.net/node/view/682818/bgimage ; https://bugguide.net/node/view/817340/bgimage
	<i>Thionia bullata</i> (Say)	Wilson and McPherson 1979; Wheeler and Wilson 1988; * https://bugguide.net/node/view/213562/bgimage ; * https://bugguide.net/node/view/528157/bgimage
	<i>Thionia elliptica</i> (Germar)	Wheeler and Wilson 1987
	<i>Thionia quinquata</i> Metcalf	* https://bugguide.net/node/view/977752/bgimage
Tropiduchidae	<i>Neaethus maculatus</i> Melichar	* https://bugguide.net/node/view/1411541/bgimage

the bright white wax that covers the caterpillar and characteristic wing dysplasia, but the wings of non-dysplastic specimens were also physically separated by hand to verify the absence of the parasite. Approximately 50–60 *A. conica* were observed, along with ~10–15 *A. bivittata* (Say), 5–10 *Flatormenis proxima* (Walker), and 5–10 *Metcalfa pruinosa* (Say); whereas *Ormenoides venusta* (Melichar) is known from the park, no individuals were encountered during our search efforts. Only *A. conica* was observed with the parasite, with six individuals noted as parasitized, five at Ojibway Park and one at Ojibway Prairie Provincial Nature Reserve. The individuals at Ojibway Park were all taken near the site of the original observation, so may all be from a single egg-laying event. All observed larvae were retained for study; however, despite the caterpillars pupating, adult moths were not successfully reared. Five pupae were retained and deposited at the University of Guelph Insect Collection (DEBU) in Guelph, Ontario, Canada. Subsequently, JV, TP, and GP observed adults at Brunet Park, LaSalle, Ontario, on 22 August (Fig. 4), 26 August, 30 August, and 3 September 2020 (Table 1). The 25 August specimen was retained by BC, and

the three adults observed by TP were deposited at DEBU. GP visually surveyed another local site, Devonwood Conservation Area, LaSalle, at night on 4 September 2020 using targeted surveys of foliage with a flashlight, which proved to be an effective method to locate the pupa based on the contrasting bright white colouration. Approximately 45–50 pupae were observed, including both hanging from silken threads of varying length (Fig. 5) and directly attached to leaves or branches (Fig. 6).

A review of the scientific literature and searches through the web portals BugGuide (<https://bugguide.net/>) and iNaturalist, where posted images of both host species and *F. exigua* caterpillars were examined, were carried out to provide an updated list of known hosts (Table 2) to aid in further searches. Several hosts were unidentified in the posts and are identified here (indicated by “**”) using Bartlett (2020) and Doering (1938). The current hosts all fall within the families Acanaloniidae, Flatidae, Issidae and Tropiduchidae, which comprise all of the ‘higher Fulgoroidea’, except for Caliscelidae (Song and Liang 2013), which is the only family of higher Fulgoroidea that remains to be reported as a host, perhaps due to their comparatively small body size. Surprisingly, *A. bivittata* is not a known host, despite it being one of the most commonly observed planthoppers in northeastern North America, including the Windsor area, where it is abundant.

The Ojibway Prairie complex has been heavily sampled historically (e.g., Paiero *et al.* 2010), and directed efforts on certain taxa continue to find new additions to the site species list, so it is not unusual to find new records in the area; however, based on previous extensive search efforts in the area, we argue that *F. exigua* has only recently become established in Ontario. The area was sampled for Lepidoptera by John E. Pilkington (between 1974 and 1985) and, more recently, over 56 opportunistic night surveys have been conducted since 2014 (TP pers. obs.), using high powered mercury vapour or metal-halide lights to photo document moth and other invertebrate species in the “Biodiversity of Ojibway Prairie Complex” iNaturalist project, with 335 Lepidoptera species reported for the site (Ojibway Nature Centre 2021). Hemipterans were actively surveyed in Paiero *et al.* (2010). Additionally, GP has been actively photographing various arthropods in and around the Ojibway Prairie complex since 2019, both during the day and night, including numerous *A. conica* specimens in 2019 and 2020. Despite the sum of these efforts, it was not until 7 August 2020 that the first *F. exigua* was found. In 2020, a total of 20 light trapping efforts from 4 June to 23 September yielded four adults of *F. exigua*. Based on the extent of the effort to document both the host species and Lepidoptera in the complex during its active period, it is likely that *F. exigua* has only recently arrived in Ontario, possibly due to the movement of its hosts. Several of local planthopper species have expanded their range further into the province over the past few decades. Previously, *Acanalonia conica*, *Flatormenis proxima* and *Ormenoides venusta* were largely restricted to extreme southwestern Ontario, with the latter two only recently recorded in Canada (Paiero *et al.* 2003), but they have now expanded their range into the Greater Toronto Area and beyond based on recent iNaturalist observations. Pechuman and Wheeler (1981) noted that *A. conica* had been expanding its range northward for many decades and appears to continue to do so; whereas *A. conica* has been established in Ontario for a longer period (the earliest known record is 1985 from Windsor), it too appears to have expanded into eastern Ontario and Quebec. With the apparent range expansion of its hosts, it is possible that *F. exigua* will soon be more widely distributed in southern Ontario.

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